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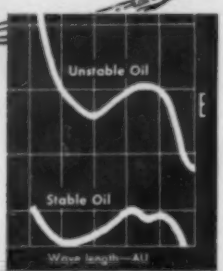

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Some Aspects of the Pharmacology of Ethyl-4-phenyl-1-[2-(phenylamino)ethyl]-piperidine-4-carboxylate (Win 13,797)*

By CHARLES BERRY†, ALAN BURKHALTER‡, LESTER W. DAVIS, E. G. GROSS, and H. H. KEASLING

The pharmacological activity of an analog of meperidine has been compared with morphine and meperidine and its effects evaluated when tested for toxicity, on toothpulp thresholds, respiration, the gastrointestinal tract, and circulation. It is concluded that Win 13,797 is much more active than morphine on a milligram basis but otherwise is qualitatively similar.

THE MANY side effects of presently available potent analgetics have prompted the continuing search for new agents. The substitution of phenylamino ethyl for methyl on the nitrogen atom of meperidine yields a material which seemed to have promise as an analgetic. This report details a pharmacologic survey of the activity of this compound: ethyl-4-phenyl-1-[2-(phenylamino)ethyl]-piperidine-4-carboxylate (Win 13,797), the structure of which is shown in Fig. 1.

EXPERIMENTAL

Acute Toxicity.—The intravenous LD_{50} was determined in mice weighing about 30 Gm. Groups of 10 mice were utilized at each dose. The LD_{50} and the 95% confidence limits were calculated by the method of probits as described by Finney (1).

Toothpulp Thresholds.—The elevation of thresholds following electrical stimulation of the toothpulp in rabbits receiving doses of morphine or Win 13,797-3 was measured by the method of Yim, *et al.* (2).

Respiration.—(*a*) Minute volumes were determined simultaneously during the experiments on toothpulp thresholds as previously described by Yim (2).

(*b*) pCO_2 Sampling: Rabbits were anesthetized with ether and polyethylene cannulas were inserted into the trachea and tied firmly in place. The skin incision was closed with wound clips and the animals were allowed to recover for at least four hours. The pCO_2 of the expired air was sampled via a polyethylene catheter leading to an LB-1 infrared CO_2 gas analyzer. When stable control levels were obtained drugs were administered subcutaneously and the end-expired pCO_2 sampled at intervals of fifteen, thirty, forty-five, and sixty minutes.

(*c*) Placental transmission: Pregnant rabbits were anesthetized by the spinal administration of lidocaine hydrochloride (1%) one or two days before term. A laparotomy was performed and the uterus exposed. In a modification of the technique of Halasey and Dille (3) one or two fetuses were removed to serve as controls and intravenous drug administered to the mother. Five minutes later the remaining fetuses were delivered and respiratory rate counts were made at five-minute intervals.

Gastrointestinal Tract.—(*a*) The inhibition of the passage of a charcoal meal was determined by the technique of Macht and Barba-Gose (4). Four young, mature rats were used at each dose.

(*b*) The activity of the stomach and intestine of the awake dog was recorded by means of water-filled balloons inserted in a stomach fistula and a Thiry-Vella loop. The dogs were trained to lie quietly upon a table during the process of the recording and, following a control period, the drugs were administered subcutaneously.

* Received April 8, 1959, from the College of Medicine, State University of Iowa, Iowa City.
Presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Ann Arbor, Mich., August, 1958.
This paper is based on a thesis submitted to the Graduate College of The State University of Iowa by C. Berry in partial fulfillment of the requirements for the degree of Master of Science.
Win 13,797 was obtained from Sterling-Winthrop Research Institute, Rensselaer, N. Y.
† Public Health Service Research Fellow of the National Institutes of Health.
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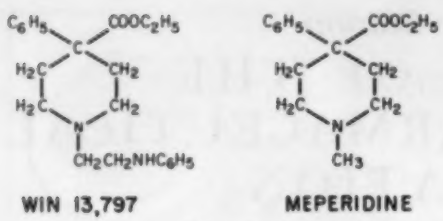

Figure 1.

(*c*) T-tubes were placed in the gall bladder of dogs anesthetized with ether. A reservoir of normal saline was arranged to supply a constant rate of perfusion of saline into the gall bladder. Recordings were made through a U-shaped manometer containing a float inserted into the perfusion circuit as described by Butsch, *et al.* (5). Forty minutes after the ether was discontinued control tracings were obtained and drugs were administered subcutaneously.

Circulation.—The arterial pressure of unanesthetized rabbits was recorded from the femoral artery. The artery was catheterized utilizing infiltration anesthesia and connected to a Statham transducer. The transducer output was amplified and recorded with an Offner Dynagraph (Type 506). Drugs were administered intravenously. The same recording system was utilized in dogs anesthetized with a mixture of 15 mg./Kg. of thiopental sodium and 250 mg./Kg. of barbital sodium.

Drugs.—Fresh drug solutions were prepared in 0.9% saline for each experiment. The materials utilized were Win 13,797-3,[1] morphine sulfate, meperidine hydrochloride, nalorphine hydrochloride, epinephrine chloride, levarterenol bitartrate, and acetylcholine chloride.

RESULTS AND DISCUSSION

The appearance of animals receiving Win 13,797-3 is qualitatively similar to that of animals treated with morphine. With sublethal doses of morphine sulfate or Win 13,797-3 in mice the Straub tail phenomena was characteristic. Following larger doses of morphine, mice exhibit a short excitement stage followed by clonic and tonic convulsions from which death may ensue. Following larger doses of Win 13,797-3 the same series of effects may be observed although the onset of effect may be more rapid. In qualitative studies in rabbits high doses of Win 13,797-3 produced convulsions which were indistinguishable from those induced by morphine. The intravenous LD_{50} in mice of morphine was determined to be 180 mg./Kg. with 95% confidence limits of 90–210 mg./Kg. while the LD_{50} of Win 13,797-3 was 22 mg./Kg. with 95% confidence limits of 20–25 mg./Kg.

Toothpulp thresholds and respiratory minute volumes were determined by administering Win 13,797-3 or morphine subcutaneously to rabbits. Changes were expressed as percentages of the control values. Figure 2 depicts a time-response curve for Win 13,797-3 at doses of 1 and 4 μM./Kg. for a time period of one hundred and fifty minutes. The upper

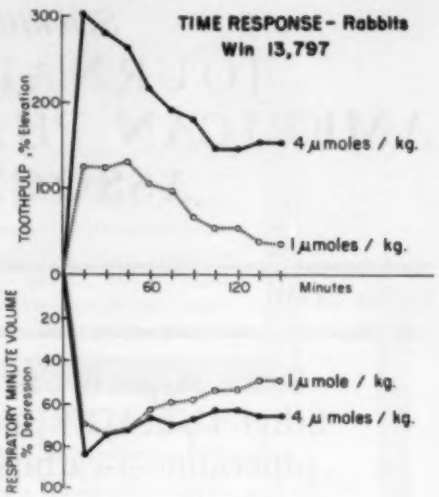

Figure 2.

curves represent percentage elevations in toothpulp thresholds while the lower curves represent percentage depression of respiratory minute volumes. It will be noted that a peak of effect is seen fifteen to thirty minutes after drug administration. In contrast, morphine sulfate peak effects are noted forty-five to sixty minutes after drug administration. In other respects the time-response curves for morphine and Win 13,797-3 at equieffective doses are essentially similar.

The relative potency of Win 13,797-3 and morphine on a molar basis was determined using toothpulp thresholds in a 4-point parallel line assay after the method described by Finney (1). With regard to the increase in toothpulp threshold Win 13,797-3 is 19 times as active as morphine. The fiducial limits at the 95% level are 14 and 27.

Recent investigations in our laboratory have elucidated the oral efficacy of morphine upon the rabbit toothpulp threshold. The ratio of effectiveness was compared using the 4-point parallel line assay of Finney (1). Administration of subcutaneous morphine was 9 times as effective as orally administered morphine with fiducial limits at the 95% level of 6–13. Attempts to compare the oral effectiveness of Win 13,797-3 with the subcutaneous doses showed that even at oral doses 100 times as high as the hypodermic dose the activity of the oral dose was substantially lower. Further consideration of this approach did not appear fruitful.

The antagonism of Win 13,797-3 by nalorphine was studied utilizing simultaneous subcutaneous administration. Mixtures of equal amounts, $^1/_5$, $^1/_{10}$, and $^1/_{25}$ the molar quantities of nalorphine with a fixed 2 μM./Kg. dose of Win 13,797-3 were compared. Antagonism of toothpulp threshold elevation in all ratios is significant at the 0.05 level. Respiratory minute volume depression was significantly antagonized only by the 1:1 and 3:1 ratios.

Numerous objections have been raised to the use of respiratory minute volume data as a measure of respiratory depression in man, Seed, *et al.* (6). In a series of exploratory experiments numerous attempts

[1] The material utilized exclusively in these experiments was the ethane sulfonate salt having a molecular weight of 462.6, identified as Win 13,797-3. The number Win 13,797 refers to the base.

to adapt the rebreathing technique described by these authors to the rabbit have not revealed a consistent correlation between inspired pCO_2 and alveolar ventilation. These studies are continuing in the hope of resolving this problem. Some evidence of respiratory depression was gained, however, by measuring the end-expired pCO_2. Control levels were quite variable between rabbits. While the tracings indicated that end-expired samples were being obtained it was noted that these levels varied quite dramatically with rate in control studies within the same animal. Both Win 13,797-3 and morphine, in doses producing equivalent toothpulp threshold increases, induced qualitatively similar depression of rate and increase in pCO_2 levels. The administration of intravenous nalorphine produced a prompt reversal of depression as evidenced by return of respiratory rate and pCO_2 levels to control values, in contrast to the findings reported by Lasagna (7) and Keats (8) in man.

As with morphine sulfate or meperidine hydrochloride, Win 13,797-3 is capable of passing the placental barrier and inducing respiratory rate depression in the fetus. Pregnant rabbits just before term were given, intravenously, either morphine sulfate, 4 μm./Kg.; meperidine hydrochloride, 14 μM./Kg.; or Win 13,797-3, 1 μM./Kg. Five to ten minutes after drug the respiratory rates of the drugged fetuses, when compared to the control fetuses, had decreased from $^1/_3$ to $^1/_2$. This depression was rapidly reversed by subcutaneous administration of 0.25 mg. of nalorphine to each of the drugged fetuses.

The effects of Win 13,797-3 and meperidine hydrochloride on the intestinal tract were compared by their ability to inhibit the passage of a charcoal meal in rats. The doses and per cent inhibition resulting from intraperitoneal injection of meperidine hydrochloride were: 80 μM./Kg.–6% and 160 μM./Kg.–62%. For Win 13,797-3 the values were: 1μM./Kg.–9% and 2 μM./Kg.–56%.

Qualitative gastrointestinal studies were also made in awake dogs in which Thiry-Vella loops and stomach fistulas had been previously prepared. The dogs were utilized only two days a week and subcutaneous doses of morphine sulfate, meperidine hydrochloride, and Win 13,797-3 were used. In all instances the results observed were qualitatively similar. Shortly after drug administration a prolonged rise in intestinal tone was observed accompanied by relaxation and decreased motility of the stomach. This phenomenon was usually preceded, in the case of morphine and Win 13,797-3, by spasms of the stomach musculature and the dogs were observed to retch and lick their chops periodically.

When intrabiliary pressure was measured in dogs whose gall bladder was perfused with a constant rate of flow of saline the subcutaneous administration of Win 13,797-3 resulted in an increase of pressure qualitatively similar to that observed when the dogs were injected with morphine.

Qualitative comparisons of the cardiovascular effects of Win 13,797-3 and morphine in rabbits and dogs did not reveal any significant differences between the compounds. The intravenous administration of either drug resulted in a rapid and prolonged depressor response of the blood pressure which was slow to recover. Intravenous titration with nalorphine rapidly reversed this depressor response. Blood pressure responses of small doses of epinephrine, levarterenol, and acetylcholine measured before and after the administration of Win 13,797-3 or of morphine did not differ markedly between the two compounds. Quantitative comparisons were not possible due to the confounding of the responses imposed by the prolonged depression of the blood pressure.

Tracings of the blood pressure of normal awake rabbits showed that there was immediate reflex compensation of the blood pressure when the animals were tilted at a 45° angle from the horizontal. After the subcutaneous administration of morphine or of Win 13,797-3 the blood pressure response to tilting of the rabbit consisted of a transient pressor overshoot before reflex compensation occurred.

SUMMARY

Comparisons have been made concerning the pharmacological influence of morphine, meperidine, and Win 13,797-3. No qualitative differences were observed to exist between the compounds. Although, on the basis of toothpulp threshold comparisons, Win 13,797-3 is 19 times as effective in molar concentrations as morphine, it seems to possess no advantages over presently known analgetic compounds.

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The Evaluation of Iodinated Organic Compounds as Radiopaque Media*

By JAMES O. HOPPE†

The general properties of X-ray contrast media are discussed with particular reference to the use of iodinated organic compounds in cholecystography and urography. The development of test procedures for the evaluation of radiopaques for X-ray visualization of the gallbladder and the upper urinary tract is described. Relative oral cholecystographic activity, from most to least active, was observed in the cat as follows: iopanoic acid, iophenoxic acid, iodoalphionic acid, and iodophthalein, in agreement with the relative order of activity reported in the literature for these compounds in man. Diatrizoate sodium was found to produce earlier and better X-ray visualization of the upper urinary tract in the rabbit with less than half the systemic toxicity observed with iodopyracet. The acute intravenous toxicity of diatrizoate sodium in the mouse, rat, rabbit, cat, and dog was found to be approximately half as great as that observed for iodopyracet, sodium iodomethamate, or sodium acetrizoate. An iodinated aromatic nucleus, to provide radiopacity, was found to be common to both cholecystographic and urographic media. The cholecystographic medium required a "transport mechanism" consisting of an alkanoic acid side chain containing 6 to 7 carbons with 2 iodines in the aromatic nucleus and 5 carbons with 3 iodines in the radiopaque portion of the molecule. The urographic contrast medium required a carboxyl group to provide high water solubility and could contain an alkanoic acid chain with no more than three carbons.

THE WORD RADIOPAQUE is used as an adjective to denote opacity to radiant energy and as a noun, synonymously with the term X-ray contrast medium, to identify specifically those substances which inhibit the passage of Roentgen or X-rays. The property of radiopacity, inherent in a variety of chemical elements, has increased immensely the boundaries of the science of diagnostic radiology. It was apparent almost from the moment of Roentgen's discovery of the X-ray in 1895 (1) that here was an important new medical tool which was capable of producing an image on photographic film corresponding to the morphology of the bony tissues but not of the soft tissues of the body. Radiopaque media have made it possible for physicians to examine the anatomy and observe the physiology of the stomach, gallbladder, urinary tract, heart, blood vessels, lungs, and other soft tissue structures without having to resort to exploratory surgery. These substances are important diagnostic aids to the radiologist in enabling him to establish the presence or absence of disease in various organs within the body. It is the purpose of the present communication to discuss briefly the general properties of radiopaques and, in particular, the development of test methods for evaluating iodinated organic compounds as cholecystographic and urographic media.

* Received May 4, 1959, from the Pharmacology Section, Sterling-Winthrop Research Institute, Rensselaer, N. Y.
Presented to the Scientific Section of the A.PH.A., New York meeting, May 3, 1957.

† Recipient of the Ninth (1957) Chilean Iodine Educational Bureau Award in recognition of his development of quantitative laboratory methods for evaluating iodine-containing diagnostic drugs and his many other contributions to the study of iodine-containing compounds.

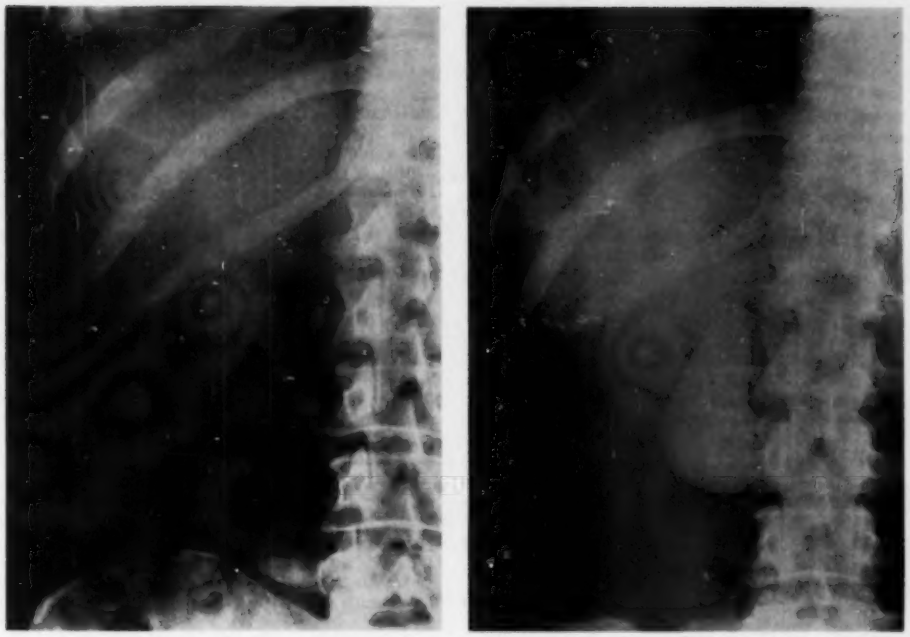
GENERAL PROPERTIES OF RADIOPAQUES

Function of a Radiopaque.—The use of a radiopaque might be illustrated with the aid of the two X-ray pictures reproduced in Figs. 1A and 1B. Figure 1A shows an X-ray view of the upper right quadrant of the human abdomen. The ribs, lumbar vertebrae, and crest of the ilium are clearly visible. Also present in this same area are the gallbladder, right kidney, blood vessels, and portions of the stomach, liver, and intestines, but these structures cannot be seen. Figure 1B shows an X-ray view of the same area in the same patient some twelve hours after the oral ingestion of an iodinated organic compound specifically designed to localize in the bile in the gallbladder. The presence of the radiopaque (iopanoic acid, U. S. P., Telepaque[1]) in the bile prevented the passage of the X-rays during the exposure to leave an image on the film corresponding to the morphology of the normal human gallbladder which appears as a white, pear-shaped mass near the center of Fig. 1B.

Physical Properties.—One might ask why the bony tissue was visible whereas the soft tissue structures were not. Further, why did the radiopaque cause the gallbladder to become visible in Fig. 1B? Since the image on the X-ray film depends upon the amount of X-rays which pass through the subject, the answers to both questions can be found with the aid of the data in Table I.

The formula states that the ability of an element to absorb X-rays (μ) varies according to the third power of the wavelength (λ) and the fourth power of the atomic number (Z). The value "k" is a constant and the value "0.2" is a factor to account for the scattering effect depending upon the wavelength at which the measurements are made. It is readily apparent that the absorption of X-rays increases enormously as the atomic number goes up. The soft tissue structures of the body, composed of carbon, hydrogen, oxygen, and nitrogen with low atomic

[1] Product of Winthrop Laboratories, New York, N. Y.



A B

Fig. 1.—Cholecystography in man. A. X-Ray picture before cholecystographic contrast medium. B. X-ray picture at twelve hours after oral ingestion of iopanoic acid. (X-ray pictures furnished by Dr. William Shehadi, New York Polyclinic Hospital, New York, N. Y.)

TABLE I.—ABSORPTION OF X-RAYS IN RELATION TO ATOMIC NUMBER

$$\mu = k\lambda^3 Z^4 + 0.2 \qquad (2)$$

Element	Atomic Number
Hydrogen	1
Carbon	6
Nitrogen	7
Oxygen	8
Phosphorus	15
Calcium	20
Bromine	35
Iodine	53
Barium	56
Mercury	80
Lead	82
Thorium	90

numbers, appear on the X-ray film as an undifferentiated mixture of black and gray shadows. In contrast, the bony tissues, composed of calcium and phosphorus with considerably higher atomic numbers, appear as sharply defined white areas on the X-ray film.

It would appear, therefore, that the ideal contrast medium would incorporate an element with a very high atomic number. Proceeding down the list of elements in Table I to thorium, with an atomic number of 90, one might assume that this element would make an excellent X-ray contrast medium. Indeed, it was used in the form of a thorium dioxide sol for visualizing blood vessels but was later found to possess two serious disadvantages: (*a*) it is retained indefinitely in the body after intravenous injection and (*b*) thorium is radioactive (3, 4). Even more disconcerting, thorium has a half-life of 1.34×10^{10} years (5). Lead and mercury have defied the efforts of the chemist to synthesize acceptable molecules containing these elements to absorb X-rays. They do not form a stable carbon bond and, hence, are not safe from the hazards of heavy metal poisoning when used in soluble form in the large dosages necessary for X-ray contrast visualization. Barium, in the form of insoluble barium sulfate, is one of the oldest useful radiopaque elements used for gastrointestinal visualization but possesses the same disadvantages of lead and mercury in the soluble form. The two halogens, bromine and iodine, both form firm carbon bonds and can be incorporated into a wide variety of organic structures permitting high water solubility and low toxicity. Although it possesses a lower atomic number, bromine absorbs X-rays more effectively than iodine in the longer wavelength of the X-ray spectrum (0.4 to approximately 1.0 Å.). Iodine, on the other hand, absorbs X-rays much more effectively than bromine in the shorter wavelength (0.2 to approximately 0.4 Å.). The long wavelength X-rays are not useful in producing an X-ray image of soft tissue structures because of lack of penetration and the greater hazard of radiation injury. This, then, narrows the field down to the element iodine at the present time. Iodine is the most widely used element in the designing of radiopaques for two important reasons: (*a*) it will form a firm carbon bond and (*b*) this property enables the chem-

ist to incorporate iodine into many different types of organic structures so that it can be administered safely in the large quantities necessary for the X-ray visualization of soft tissue structures.

Biological Properties.—From these considerations, it is possible to set forth the essential biological properties which a compound must possess in order to be acceptable as a radiopaque.

High Radiopacity.—Radiopacity is an obvious requirement in order to classify the compound for pharmacological evaluation. High radiopacity is listed under biological properties because the greater the ability of a compound to inhibit the passage of X-rays, the less of the material will be required, thus providing greater specificity of action.

Low Systemic and Local Tissue Toxicity.—Low toxicity, both at the systemic and local tissue levels, is extremely important because of the massive dosages used and the conditions under which these doses are administered. For example, in visualizing the chambers of the heart, a volume of 50 cc. of a 50 to 90% aqueous solution may be injected intravenously in two seconds or less.

Pharmacodynamically Inert.—In most instances, the patients who receive compounds of this type are not in the best state of health. The radiopaque should not aggravate their condition by causing hypotension, vomiting, nausea, dizziness, diarrhea, headache, etc. The critical demands upon a radiopaque compound are contrary to the usual pursuit of the pharmacologist who looks for ways and means of increasing pharmacological activity. In the case of the radiopaque, it is essential that it be pharmacodynamically inert.

Selective Localization.—The gastrointestinal tract can be readily visualized by swallowing a barium sulfate meal. It is necessary to resort to indirect methods in order to visualize the less readily accessible structures as the gallbladder or the upper urinary tract. The radiopaque should be capable of being transported in the blood to the specific organ in question and there concentrated in the normal tissue fluid to cause the soft tissue structures to visualize on X-ray film.

Prompt and Complete Elimination.—Once the X-ray pictures have been taken, there is no longer any need for the presence of the radiopaque and, therefore, it should be promptly and completely eliminated with no discomfort to the patient.

CHOLECYSTOGRAPHY

Cholecystography is the roentgenographic examination of the gallbladder with the aid of an X-ray contrast medium. Calcified stones in a gallbladder would be visible in the ordinary X-ray picture as in Fig. 1A without the aid of an X-ray contrast medium. The great majority of gallstones, however, are composed of cholesterol or a mixture of cholesterol and bile pigments. The elemental composition of these stones is similar to that of the tissues of the gallbladder itself and, consqeuently, are not visible in the ordinary X-ray picture. Gallstones of this type could have been present in Fig. 1A. The absence of dark spots within the gallbladder in Fig. 1B indicates that these radiolucent gallstones were not present.

Cholecystography was discovered by the late Dr. Evarts Graham in 1924 (6–12). One evening in 1922, while contemplating the pharmacological observations of Abel and Rowntree (13) that phenoltetrachlorphthalein was eliminated almost entirely in the bile, Dr. Graham conceived the idea of replacing the chlorine with other atoms with greater radiopacity to obtain an X-ray shadow of the gallbladder (12). It could be said that cholecystography was discovered by accident. The sodium salt of tetraiodophenolphthalein was injected intravenously into six dogs. A faint gallbladder picture became visible in only one of the six dogs. It was discovered later that the five dogs which did not show a gallbladder picture had been fed on the morning of the experiment, whereas the one dog which did show a gallbladder shadow had been accidentally overlooked. The severe toxic reactions encountered with tetraiodophenolphthalein caused Dr. Graham to turn to tetrabromphenolphthalein which was found to be better tolerated. Dr. Graham's first published pictures of the gallbladder in the dog and in man were made with tetrabromphenolphthalein (6). The earlier toxic reactions with tetraiodophenolphthalein were found to be due to impurities which were eventually eliminated, thus making it possible to use the more radiopaque halogenated phenolphthalein.

Tetraiodophenolphthalein was given in the form of the sodium salt (iodophthalein sodium, U. S. P., Iodeikon[3]) as a bright blue solution by either intravenous injection or oral administration. Administered orally, its taste was nauseating. If the patient was able to keep it down, much of the dose was lost through diarrhea. It was more frequently administered intravenously in order to circumvent the bitter taste and inaccuracy of dosage due to vomiting and diarrhea. Even though it was administered by slow intravenous infusion, the incidence of nausea and vomiting still remained high. In spite of the frequency and intensity of side effects, iodophthalein remained the cholecystographic contrast medium of choice for nearly twenty years.

The first real improvement in cholecystography resulted from the studies of Dohrn and Diedrich (14), Kleiber (15), and Junkmann (16) in Germany in 1940 with the compound β-(3,5-diiodo-4-hydroxyphenyl)-α-phenyl propionic acid, which they called Biliselectan.[3] Shortly thereafter, this compound was introduced into this country with the name iodoalphionic acid, U. S. P., or Priodax.[4] Although there was no great reduction in nausea and vomiting, it rapidly replaced iodophthalein in cholecystography largely because it could be administered simply in tablet form with greater convenience for both the radiologist and the patient (17–23).

Perhaps by tradition, the dog had become the chosen animal for the experimental investigation of compounds designed for cholecystography. In 1946, when I first became interested in this field, Epstein, *et al.* (24), had just published their observations on the potential use of the frog, chick, rabbit, and dog as test animals for cholecystographic media, and concluded that the dog was the most satisfactory of this group. However, they pointed out, and later emphasized (25), that compounds which gave the best results in the dog did not necessarily give the

[3] Product of Mallinckrodt Chemical Works, St. Louis, Mo.

[3] Product of Schering, A.G., Berlin, Germany.

[4] Product of Schering Corporation, Bloomfield N. J.

best gallbladder shadows in man. Jones, *et al.* (26), and later, Margolin, *et al.* (27), considered the dog the most satisfactory of the laboratory animals for the study of cholecystographic media. Our own investigations indicated that the mouse was too small, the rabbit and guinea pig gave inconsistent shadows, and the cat and dog gave reasonably consistent gallbladder shadows. Since there was no previous evidence to indicate that earlier investigators had tried the cat, it was decided to explore more fully the use of this species, in view of the reported discrepancies in the dog data (28). It was found that the cat was capable of discriminating among small changes in dosage and when the effect, in terms of density of the gallbladder shadow, was plotted against the logarithm of the dose, the relationship was linear in nature, thus satisfying the basic requirements of a biological assay (28–30). The cat offered an even more practical advantage over the dog in that its weight was about one-fifth that of the dog so that approximately five times as much information could be obtained from the same amount of test compound.

Method.—The details of the test procedure using the cat have been described previously (30). The interpretation of the cholecystogram and the assignment of an adjective rating in terms of a numerical equivalent, called the Cholecystographic Index (CI), is illustrated in Fig. 2. After each film is read, the CI values are averaged for a given dose level and designated the Average Cholecystographic Index (ACI) for that dose.

Experimental.—The use of this test method in the study of three different series of compounds is illustrated in Fig. 3. In series A, the compound with R = hydrogen, is iodopyracet, U. S. P., or Diodrast,[5] which is a well-known intravenous urographic contrast medium. This compound failed to produce any roentgenographic evidence of gallbladder visualization at 100 mg./Kg. intravenously in the cat. With R = methyl, very faint evidence of gallbladder concentration became apparent. When R = propyl, the gallbladder and urinary bladder appeared with almost equal intensity, indicating approximately an equal distribution between urinary and biliary excretion. Maximum gallbladder visualization was reached when R = butyl, or a total of six carbons in the alkanoic acid side chain. The density of the gallbladder shadows decreased gradually with R = amyl and hexyl, and dropped sharply at R = heptyl. The acute intravenous toxicity of this series of compounds in the mouse remained at about 6 Gm./Kg. with R = hydrogen and methyl, doubled at R = propyl, and continued to double with the addition of each methylene group, so that by the time fair to good gallbladder visualization was obtained, acute toxicity had increased to a point where it was likely to interfere with the clinical use of these compounds by this route of administration.

Turning to oral administration, it was found that a similar relationship existed between gallbladder visualization and chemical structure. More important, the intensity of the gallbladder shadow at a given dose level following oral administration appeared to be the same as that obtained by intravenous injection. Although the rate of appearance of the gallbladder shadow was slower in some instances by the oral route, this was offset by a marked reduction in systemic toxicity. The similarity between chemical structure and gallbladder visualization in the cat by both intravenous and oral administration is illustrated by series A and B in Fig. 3. Observations on more than 100 diiodo compounds indicated that a cholecystographic contrast medium consisted of two essential parts: (*a*) a firmly bound iodine-bearing nucleus to provide radiopacity and (*b*) a side chain containing a carboxyl group and an optimal size lipophilic group to provide for absorption and to direct excretion into the bile (28–34). Gallbladder visualization in the cat was obtained when the number of carbons in the side chain ranged from three to eight carbons in agreement with the findings of Epstein, *et al.* (24), in the dog. It was found that an iodinated phenyl nucleus could contain an amino group, a hydroxyl, or no substituent other than the iodines (32). The phenolic hydroxyl, which occurs in iodophthalein and iodoalphionic acid, was not found to be essential for gallbladder visualization as suggested by Epstein, *et al.* (24), and Natelson, *et al* (35). The nature of the side chain appears to be the most critical factor in determining whether gallbladder or urinary bladder visualization predominates. Where the alkanoic acid side chain contained less than three carbons, excretion occurred primarily by way of the kidneys. When the length of the side chain exceeded seven carbons, the compound tended to remain unabsorbed in the gastrointestinal tract. As each of several series of diiodo compounds was studied, maximum gallbladder visualization was found to be equal to but not much better than that observed with the diiodo reference compound, iodoalphionic acid, U. S. P.

Efforts to improve visualization by changes both in the radiopaque nucleus and in the side chain had not been successful. It occurred to me that perhaps the solution to this problem might be as simple as adding a third bulb to a light fixture. If the same number of molecules could be concentrated in the bile using a compound containing three iodine atoms instead of two, the triiodo compound should produce a brighter gallbladder picture. Among the various test compounds was one containing three iodine atoms. This compound, the ethyl-substituted triiodo structure shown in series C in Fig. 3, had been synthesized by Mr. T. R. Lewis and Dr. Sydney Archer of the Organic Chemistry Division of the Sterling-Winthrop Research Institute (36). To test this hypothesis, I took a 3-Gm. dose in the evening with a fat-free meal. After X-ray the next morning, I was elated to find that, indeed, the density of my own gallbladder shadow was distinctly greater than that obtained in earlier studies with diiodo compounds. In the cat, this compound produced good to excellent visualization of the gallbladder in comparison with fair to good visualization with the best of the diiodo compounds. As both the lower and higher members of this series were prepared and tested, they were found to distribute themselves in the form of a pyramid with the original ethyl derivative as the most effective compound for visualizing the cat gallbladder (see series C, Fig. 3). In contrast to the findings among the diiodo compounds, peak visualization was observed in the 3-amino-2,4,6-triiodophenyl alkanoic acid series with a

[5] Product of Winthrop Laboratories, New York, N. Y.

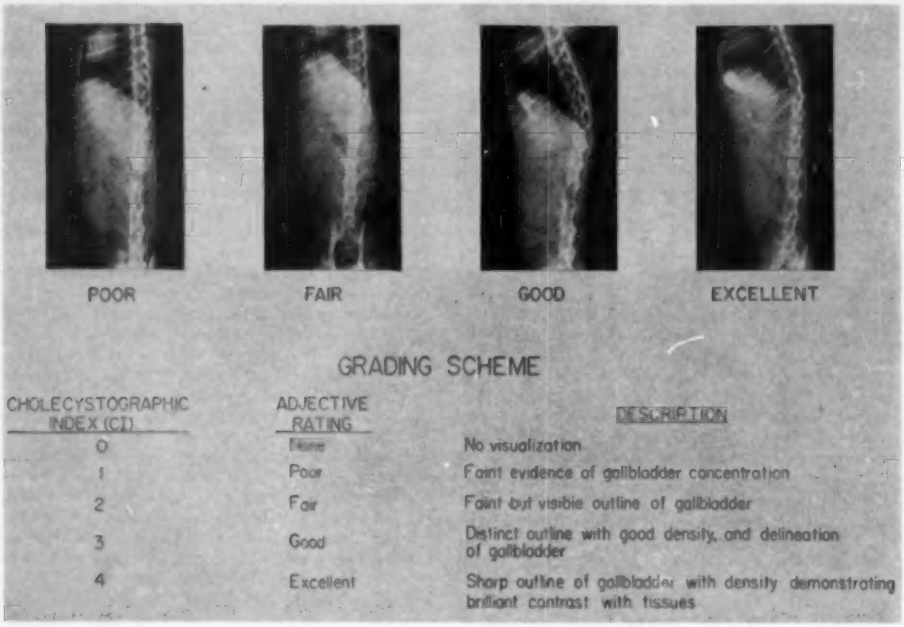

POOR
FAIR
GOOD
EXCELLENT
GRADING SCHEME
CHOLECYSTOGRAPHIC INDEX (CI)
ADJECTIVE RATING
DESCRIPTION
0 None No visualization
1 Poor Faint evidence of gallbladder concentration
2 Fair Faint but visible outline of gallbladder
3 Good Distinct outline with good density, and delineation of gallbladder
4 Excellent Sharp outline of gallbladder with density demonstrating brilliant contrast with tissues

Fig. 2.—Cholecystographic screening procedure in the cat.

total of five carbons in the side chain. On the basis of these observations, the ethyl triiodo derivative was selected for further study as a potential cholecystographic contrast medium which later became known as iopanoic acid, U. S. P., or Telepaque.[1]

Cat *vs.* Dog.—Still unanswered was the question as to whether the cat or the dog provided an answer which could be relied upon in predicting which compounds might be useful in visualizing the gallbladder in man. The ACI for iopanoic acid was determined in both the cat and dog in comparison with iodophthalein, iodoalphionic acid, and iophenoxic acid[6] with results as summarized in Table II.

The structural formulas of these compounds and their respective dose response curves are shown in Fig. 4. Iodophthalein was clearly the least effective of the four compounds in visualizing the gallbladder in both species. Interestingly enough, it was distinctly more effective in visualizing the gallbladder of the dog than it was in the cat. For convenience in analyzing the data, a value indicating good visualization of the gallbladder, ACI_3, was derived from each of the curves except that for iodophthalein which was too ineffective. The ACI_3 values in the dog indicated the following order of activity, from most to least active: iophenoxic acid, iopanoic acid, and iodoalphionic acid. The ACI_3 values in the cat, on the other hand, indicated that iopanoic acid was the most effective, iodoalphionic acid the least effective, and the activity of iophenoxic acid was intermediate between these two. The early clinical studies on iopanoic acid indicated that the density of the human gallbladder shadow was distinctly greater and the incidence of side effects less than

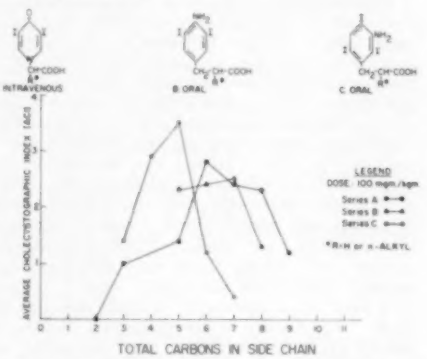


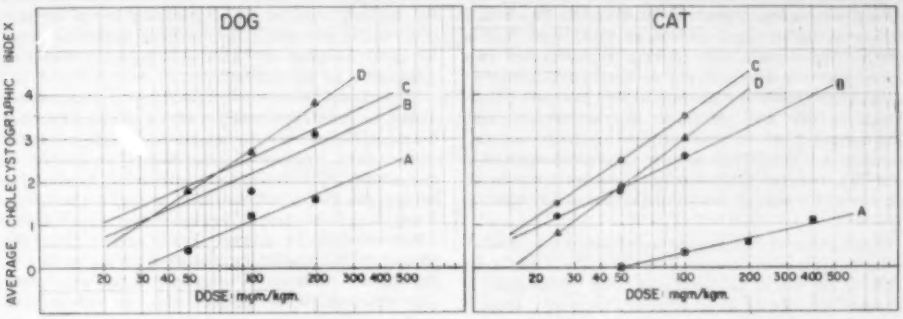
Fig. 3.—The relationship between chemical structure and gallbladder visualization following intravenous and oral administration of iodinated organic compounds in the cat.

that observed with iodoalphionic acid (37–54). Morgan and Stewart (40) observed that the density of the gallbladder shadow with iopanoic acid in man was approximately 1.35 times as great as that observed with iodoalphionic acid. This figure is in remarkably close agreement with the initial value, derived from the ACI's for these two compounds in the cat, which indicated that iopanoic acid was approximately 1.4 times as effective as iodoalphionic acid in visualizing the cat gallbladder (29). Iophenoxic acid, described by Dr. Domenic Papa, a previous recipient of this award, and his associates (55, 56) was found, in man, to produce gallbladder shadows which were less dense than those observed

[1] Product of Schering Corporation, Bloomfield, N. J.

TABLE II.—ORAL CHOLECYSTOGRAPHY IN THE CAT AND DOG

Species	Dose, mg./Kg.	—Iopanoic Acid— No. of Animals	ACI	—Iodophthalein— No. of Animals	ACI	Iodoalphionic Acid No. of Animals	ACI	Iophenoxic Acid No. of Animals	ACI
Cat	25	106	1.5	..	..	40	1.2	40	0.8
Cat	50	108	2.5	5	0	40	1.8	40	1.9
Cat	100	115	3.5	20	0.35	40	2.6	40	3.0
Cat	200	..	..	20	0.6	..	..	..	..
Cat	400	..	..	15	1.1	..	..	..	..
Dog	50	6	1.8	5	0.4	5	1.8	5	1.8
Dog	100	7	2.7	5	1.2	8	1.8	6	2.7
Dog	200	7	3.1	5	1.6	8	3.1	6	3.8



COMPOUND	DOG ACI3 mgm/kgm	DOG RELATIVE ACTIVITY	CAT ACI3 mgm/kgm	CAT RELATIVE ACTIVITY
Iopanoic Acid	157	1.0	70	1.0
Iodoalphionic Acid	230	0.68	155	0.45
Iophenoxic Acid	117	1.34	100	0.70

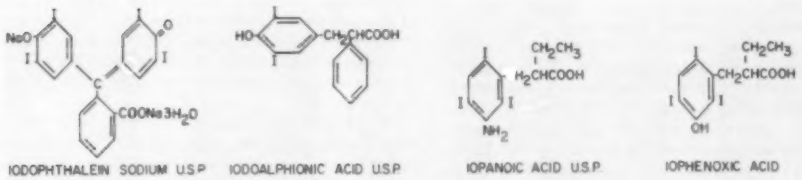


Fig. 4.—A comparison of the oral cholecystographic activity of iodophthalein, iodoalphionic acid, iopanoic acid, and iophenoxic acid in the cat and dog.

with iopanoic acid, and intermediate in density between those of iopanoic acid and iodoalphionic acid (57–60). It can be concluded, therefore, that the cat proved to be a more reliable test animal than the dog for predicting the effectiveness of these compounds in visualizing the human gallbladder.

A frequent reminder of the early work with iodophthalein in cholecystography is the use of the word "dye" synonymously with the term X-ray contrast medium. It is true that iodophthalein possesses dye properties, but this fact has nothing to do with its ability to produce an image on X-ray film. None of the newer radiopaques are dyes. The use of the word dye in place of radiopaque or X-ray contrast medium to describe them is incorrect and should be discontinued.

UROGRAPHY

Urography is the roentgenographic examination of the urinary tract with the aid of an X-ray contrast medium. Retrograde urography involves the instillation of the radiopaque into the urinary tract by way of the urethra. Excretion urography involves the intravenous injection of a radiopaque which is rapidly excreted in the urine in sufficient concentration to cause the urinary tract to become visible on X-ray film. The present discussion is concerned only with excretion urography.

The early concepts leading to the use of iodinated organic compounds for excretion urography originated in Europe at approximately the same time as the discovery of cholecystography. In 1921, Professor Arthur Binz of Berlin, Germany, conceived the idea of synthesizing heterocyclic compounds containing iodine or arsenic to improve the therapeutic efficiency of the treatment of syphilis (61). Professor Binz and his assistant, Dr. Räth, synthesized some 700 new arsenic and iodine compounds, some of which were found to have a surprisingly low systemic toxicity. One of these, N-methyl-5-iodo-2-pyri-

done, while under investigation as a chemotherapeutic agent in the treatment of streptococcus infections, was found to be eliminated partly in the bile and, to a larger extent, in the urine (62). These observations led to the suggestion that it might be a useful X-ray contrast medium (63). Swick found that this compound produced a distinct shadow of the urinary tract but failed to visualize the gallbladder of the rabbit (63). Better tolerance and greater water solubility was achieved with sodium 5-iodo-2-pyridone-N-acetate which Professor Binz selected for investigation in excretion urography (61). This compound, called Uroselectan,[7] was found to be an effective urographic contrast medium by Swick (63, 64), von Lichtenberg and Swick (65), and Jaches (66). Uroselectan was quickly replaced by two more effective diiodo pyridone derivatives, iodopyracet (Diodrast[8]) and sodium iodomethamate (Neo-Iopax[8]) (67, 68). Sodium iodomethamate and iodopyracet, which was studied initially as the sodium salt and later as the diethanolamine salt (69–71), became the urographic contrast media of choice and remained unchallenged for nearly twenty years.

Another most fascinating suggestion for excretion urography was the proposal by Dr. Moses Swick in 1933 of the use of an organic nucleus representing a normal product of animal metabolism as a carrier for the radiopaque element necessary for the X-ray visualization of the urinary tract (72, 73). The compound suggested was sodium *ortho*-iodohippurate, a halogen derivative of a substance normally found in urine. Contrary to initial expectations, this compound (iodohippurate sodium or Hippuran[9]) did not become a widely accepted urographic contrast medium in comparison with either sodium iodomethamate or iodopyracet. Swick's idea of using a product of normal detoxication as a carrier for a radiopaque for intravenous urography lingered for many years in the imagination of Dr. Vernon Wallingford, also a previous recipient of this award, and finally in 1952 emerged in the form of a new compound, sodium 3-acetamido-2,4,6-triiodobenzoate, sodium acetrizoate or Urokon[10] (74–76). The acute intravenous toxicity of sodium acetrizoate in the mouse was less than that of either iodopyracet or sodium iodomethamate.

For many years we had been testing numerous iodinated organic compounds for acute intravenous toxicity in the mouse in an effort to find one with an acute intravenous LD_{50} value greater than the 4 to 6 Gm./Kg. level of sodium iodomethamate and iodopyracet, respectively. In 1952, this level had been raised to 8 Gm./Kg. with the discovery of sodium acetrizoate. In 1954, Dr. Aubrey Larsen of the Organic Chemistry Section at the Sterling-Winthrop Research Institute, succeeded in shattering this toxicity barrier when he described the synthesis of sodium 3,5-diacetamido-2,4,6-triiodobenzoate, diatrizoate sodium or Hypaque[11] (77–79). The acute intravenous toxicity of diatrizoate sodium in the mouse was found to be approximately 14 Gm./Kg., which indicated a substantial elevation of the systemic toxicity ceiling for compounds in the field of excretion urography. On the basis of the acute toxicity observations in the mouse, this compound was selected for further laboratory evaluation as a potential urographic contrast medium. Independently and unknown to us, this compound was described at about the same time in Germany by Langecker, *et al.* (80), and studied clinically in the form of a mixture of the sodium and N-methylglucamine salts in Germany as Urografin[12] (81, 82), and later in this country as Renografin[12] (83, 84).

Method.—The test method evolved for searching for an improved contrast medium for urography consisted of two parts: (*a*) acute intravenous toxicity determination in the mouse and (*b*) excretion urography in the rabbit.

(*a*) *Acute Intravenous Toxicity.*—As pointed out above, an arbitrary criterion of 4 to 6 Gm./Kg. had been established for the selection of compounds for evaluation in respect to experimental urography in the rabbit. Those compounds found to be effective urographic media in the rabbit in preliminary tests were then subjected to acute intravenous toxicity determinations in animal species other than the mouse, while continuing the urographic studies in the rabbit. The species used for these acute toxicity determinations included the rat, rabbit, cat, and dog. Since the acute lethal dose cannot be determined in man, it must be assumed that the systemic toxicity for man could be equivalent to the lowest LD_{50} value found among the various animal species. The compound demonstrating the highest LD_{50} value and the least variation among animal species is most likely to be the safest compound to use in man. The method for determining acute intravenous toxicity in the various species, numbers of animals used, concentrations, and rates of injection employed in the study of diatrizoate sodium in comparison with iodopyracet, sodium iodomethamate, and sodium acetrizoate have been described previously (78).

(*b*) *Urography.*—Adult, healthy, New Zealand white rabbits weighing 2.5 to 3.5 Kg. are maintained in individual cages in a well lighted, air-conditioned animal room on a nutritionally adequate diet with a maximum allowance of 500 cc. of water per day. Because of the importance of accurate timing of the serial X-ray exposures, each rabbit is studied individually. For a given test, a group of five rabbits is fasted for forty-eight hours to eliminate food shadows over the kidney area and denied access to water for twenty-four hours prior to the injection of the test compound. On the morning of the test, a control X-ray picture is taken of each rabbit to determine the radiographic status of the urinary tract area. Only those rabbits showing an abdominal field relatively free of gas and food shadows are used. The rabbit selected for testing is fastened, supine, in position under the X-ray tube. A cloth compression band is applied firmly just above the symphysis pubis immediately before injection with the first cassette in position under the rabbit. The test compound, in neutral aqueous solution, is injected intravenously at a rate of 12 cc./minute at a dose of 100, 200, 400, 800, 1,600, or 3,200 mg./Kg.

[7] Product of Schering, A.G., Berlin, Germany.
[8] Product of Schering Corporation, Bloomfield, N. J.
[9] Product of Mallinckrodt Chemical Works, St. Louis, Mo.
[10] Product of Mallinckrodt Chemical Works, St. Louis, Mo.
[11] Product of Winthrop Laboratories, New York, N. Y.
[12] Product of Schering, A.G., Berlin, Germany.
[13] Product of E. R. Squibb & Sons, New York, N. Y.

X-ray pictures are then taken at five and ten minutes after injection. The compression band is immediately released after the ten-minute picture and reapplied at ten minutes before the next X-ray pictures at twenty, forty, and eighty minutes after injection. At the higher dose levels, X-ray pictures are taken without compression at hourly intervals up to seven and twenty-four hours, and daily thereafter until evidence of complete elimination has been achieved. A minimum of five animals is used for each dose level. The same procedure is used for urographic examination in the cat and dog with the exception that fasting is limited to twenty-four instead of forty-eight hours.

The urograms are read and interpreted in accordance with the grading scheme shown in Fig. 5. The interpretation of the film is based on the ability of the compound to delineate the morphology of the upper urinary tract, the highest score being assigned when the calices, pelves, and ureters are visible as indicated in part A of Fig. 5. The quality of the shadow is also taken into consideration and assigned a numerical value as indicated in part B of Fig. 5. The X-ray pictures across the top of Fig. 5, from left to right, show the control plate before injection; visualization of the calices, pelves, and a faint outline of the upper ureters at 5 minutes; maximum visualization of these structures at ten minutes; very little radiopaque urine visible in the pelves and ureters at twenty minutes, and only a trace visible in the pelves at forty minutes, with the major portion of the injected dose visible in the urinary bladder. The urographic score is determined for each film and designated as the Urographic Index (UI). As additional animals are tested at each dose level, the urographic indexes for each time interval are averaged and designated as the Average Urographic Index (AUI). The animals are returned to the regular colony after completion of the urographic series of films and are not used again for a period of at least one week.

Experimental.—The use of this test procedure in the evaluation of the urographic properties of diatriazoate sodium in comparison with those of iodopyracet in the rabbit is shown in Table III.

Urography.—Maximum visualization of the urinary tract of the rabbit was observed with diatrizoate sodium at each dose level from 100 to 1,600 mg./Kg., at ten minutes after injection. Maximum visualization was observed with iodopyracet at ten minutes with 100 and 200 mg./Kg., at twenty minutes with 400 and 800 mg./Kg., and at ten minutes with 1,600 mg./Kg., indicating a somewhat slower rate of visualization of the urinary tract with iodopyracet. The AUI for diatrizoate sodium was greater than that observed for iodopyracet at each dose level except the 200 mg./Kg. dosage, indicating that diatrizoate sodium produced a better diagnostic picture of the urinary tract of the rabbit than iodopyracet at equivalent dose levels. It was found that dosages of 400 to 800 mg./Kg. of diatrizoate sodium and 800 mg./Kg. of iodopyracet were required in order to achieve good diagnostic visualization of the upper urinary tract in the rabbit. The AUI for diatrizoate sodium continued to increase in magnitude with increase in dosage up to 7,960 mg./Kg. where mortality began to appear. The AUI for iodopyracet, on the other hand, increased to a value of 45.0 at 800 mg./Kg., after which it showed no further important increase with increase in dosage. A 20% mortality was encountered with diatrizoate sodium at 7,960 mg./Kg. A 40% mortality was encountered with

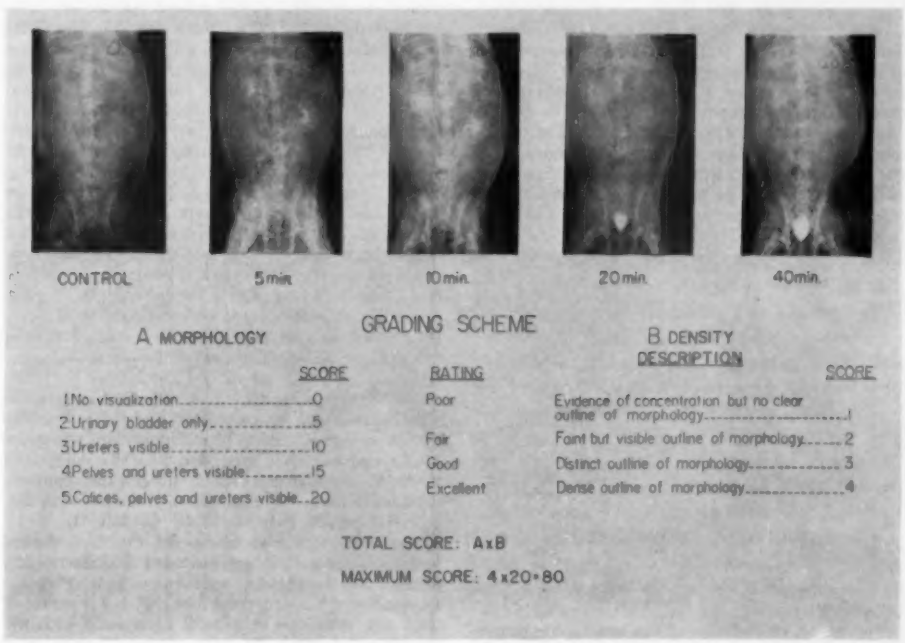


Fig. 5.—Urographic screening procedure in the rabbit

TABLE III.—INTRAVENOUS UROGRAPHIC PROPERTIES OF DIATRIZOATE SODIUM AND IODOPYRACET IN THE RABBIT

Compound	Dose, mg./Kg.	No. of Rabbits	Average Urographic Index (AUI) at Intervals After Injection, Minutes 5	10	20	40	80	120	180	240	300	360	420	Mortality
Diatrizoate sodium	100	14	21.8	29.0	21.0	15.3	9.3	..	..	..	..	..	..	0/14
Diatrizoate sodium	200	19	26.4	27.8	26.6	18.7	10.8	..	..	..	..	..	..	0/19
Diatrizoate sodium	400	17	32.6	37.0	31.2	20.6	18.2	..	..	..	..	..	..	0/17
Diatrizoate sodium	800	17	49.7	61.2	44.6	40.0	24.0	..	..	..	..	..	..	/017
Diatrizoate sodium	1,600	16	37.0	57.5	55.0	38.8	26.2	..	..	..	..	..	..	0/16
Diatrizoate sodium	3,200	21	40.5	62.6	69.6	64.0	62.5	66.8	46.0	33.3	..	..	13.3	0/21
Diatrizoate sodium	6,400	5	28.0	48.0	52.0	64.0	64.0	76.0	76.0	60.0	40.0	47.5	26.0	0/5
Diatrizoate sodium	7,960	5	36.0	44.0	50.0	50.0	57.5	70.0	80.0	70.0	50.0	25.0	25.0	1/5
Diatrizoate sodium	10,000	7	51.5	56.0	60.0	64.0	72.0	75.0	75.0	..	37.5	37.5	30.0	3/7
Iodopyracet	100	5	14.0	19.0	18.0	9.0	10.0	..	..	..	..	..	..	0/5
Iodopyracet	200	9	20.0	31.2	19.4	11.7	10.0	..	..	..	..	..	..	0/9
Iodopyracet	400	16	18.1	19.0	22.2	19.4	18.7	..	..	..	..	..	..	0/16
Iodopyracet	800	6	28.4	40.0	45.0	40.0	30.0	..	..	..	..	..	..	0/6
Iodopyracet	1,600	5	26.0	34.0	34.0	34.0	24.0	..	..	..	..	..	..	0/5
Iodopyracet	3,200	5	20.0	24.0	33.0	39.0	52.0	..	..	..	..	..	..	0/5
Iodopyracet	3,980	5	20.0	20.0	28.0	28.0	40.0	46.7	..	40.0	23.3	23.3	30.0	2/5
Iodopyracet	5,000	5	28.0	28.0	28.0	26.6	40.0	60.0	..	60.0	60.0	60.0	..	4/5
Iodopyracet	6,400	5	20.0	24.0	26.0	25.0	..	..	..	..	..	..	..	5/5

iodopyracet at 3,980 mg./Kg., indicating that diatrizoate sodium was approximately half as toxic as iodopyracet under the conditions of excretion urography in the rabbit.

An analysis of the urographic properties of diatrizoate sodium in comparison with those of iodopyracet at 800 mg./Kg. intravenously in the rabbit is illustrated in Fig. 6. The quality of morphologic delineation of the upper urinary tract increased rapidly to a maximum at ten minutes after injection of diatrizoate sodium. Visualization with iodopyracet increased more slowly to a maximum at twenty minutes after injection. The greater magnitude of the maximum urographic score with diatrizoate sodium indicates a more effective visualization of the upper urinary tract of the rabbit than was obtained with iodopyracet.

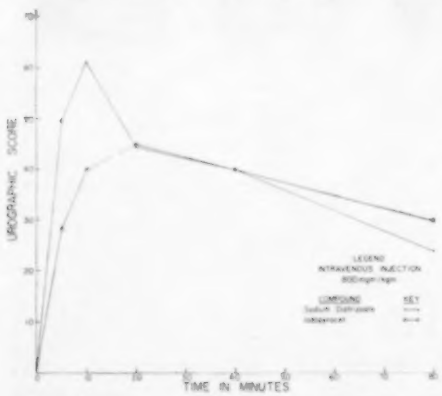


Fig. 6.—A comparison of the urographic properties of diatrizoate sodium and iodopyracet in the rabbit.

Acute Toxicity.—The acute intravenous LD_{50} values for diatrizoate sodium in comparison with those for iodopyracet, sodium iodomethamate, and sodium acetrizoate in five animal species are summarized in Fig. 7. New data included in Fig. 7 are acute intravenous LD_{50} data for iodopyracet and sodium iodomethamate in the rabbit, cat, and dog. The methods and number of animals used are identical to those described earlier (78). The acute intravenous LD_{50} for iodpyracet was found to vary from 3 Gm./Kg. to about 6 Gm./Kg., and for sodium iodomethamate from 4 Gm./Kg. to about 7 Gm./Kg. Although the acute intravenous toxicity of sodium acetrizoate was approximately 8 Gm./Kg. in the mouse, the values for the rat, rabbit, cat, and dog ranged between 5 and 6 Gm./Kg. The acute intravenous LD_{50} for diatrizoate sodium was found to range from 11.3 Gm./Kg. in the cat to 14.0 Gm./Kg. in the mouse, indicating an order of systemic toxicity in the various animal species of about half that of iodopyracet, sodium iodomethamate, or sodium acetrizoate.

The laboratory evidence of improved urographic visualization and, in particular, the marked reduction in systemic toxicity of sodium 3,5-diacetamido-2,4,6-triiodobenzoate suggested that this compound merited investigation as a urographic contrast medium in man. The first clinical studies with diatrizoate sodium, carried out by the late Dr. T. D. Moore, who had also conducted the first clinical investigations with iodopyracet in this country (70), demonstrated a distinct improvement in the diagnostic quality of the urograms along with a low rate of mild side reactions in general agreement with the experimental data in laboratory animals (85). Subsequent clinical studies indicated improved diag-

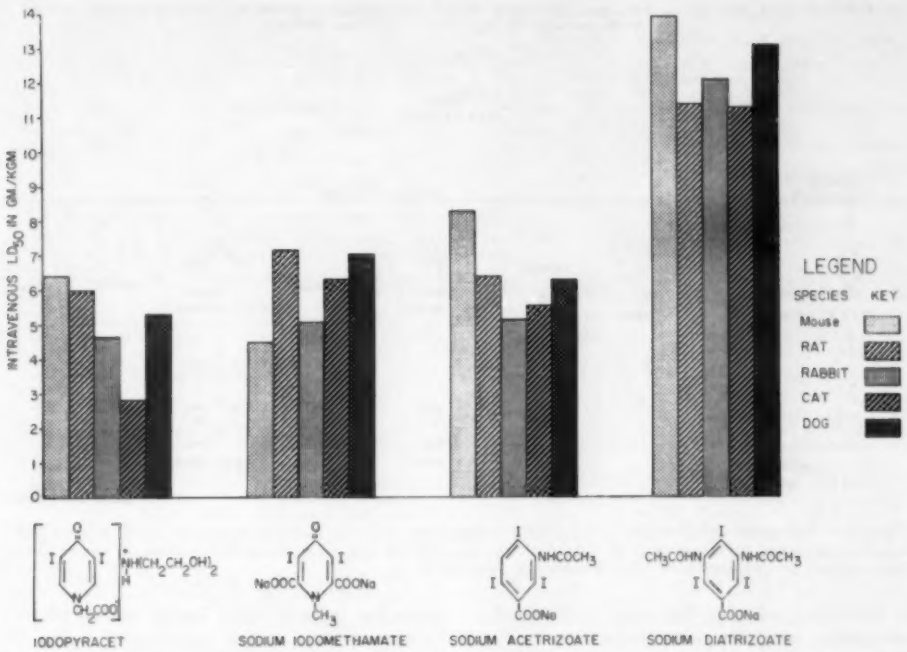


Fig. 7.—Acute intravenous toxicity data on iodopyracet, sodium iodomethamate, sodium acetrizoate, and sodium diatrizoate in the mouse, rat, rabbit, cat, and dog.

nostic visualization and a reduced incidence of side effects in comparison with iodopyracet, sodium iodomethamate, or sodium acetrizoate (86–102).

One of the most interesting observations to emerge from the laboratory studies on diatrizoate sodium was the finding that iodine could be incorporated into the sodium 3,5-diacetamidobenzoate molecule with no increase in toxicity when measured in terms of m*M*/Kg. by intravenous injection in the mouse (78). This was also found to be true in the case of sodium acetrizoate, as shown in Fig. 8. These findings suggested a possible explanation for the limited success of Dr. Swick's proposed use of iodohippurate sodium in urography (72, 73). As seen in Fig. 8, the acute intravenous toxicity of sodium benzoate in the mouse was reduced to one-half by conjugation with glycine. In contrast to the observations on the effects of iodine substitution in sodium 3,5-diacetamidobenzoate or sodium 3-acetamidobenzoate, the incorporation of iodine into sodium hippurate resulted in a twofold increase in toxicity when measured in terms of m*M*/Kg. of body weight in the mouse.

SUMMARY

1. The properties of radiopaques were discussed in general and those of iodinated organic compounds used as radiopaques in cholecystography and urography in particular.

2. The development of test procedures for the evaluation of radiopaques for cholecystography and urography were described.

3. Relative oral cholecystographic activity, in order of decreasing effectiveness, was observed in the dog: iophenoxic acid, iopanoic acid, iodoalphionic acid, and iodophthalein, and in the cat: iopanoic acid, iophenoxic acid, iodoalphionic acid, and iodophthalein. The relative order of activity observed in the cat resembled that described in the literature for man, indicating that the cat was a more satisfactory test animal than the dog for the estimation of the cholecystographic activity of these compounds.

4. The essential components of a cholecystographic medium appear to consist of (*a*) an iodinated aromatic nucleus to provide radiopacity and (*b*) a "transport mechanism" consisting of an alkanoic acid side chain containing 6 to 7 carbons with 2 iodines in the aromatic nucleus and 5 carbons with 3 iodines in the radiopaque portion of the molecule.

5. The urographic properties of diatrizoate sodium were compared with those of iodopyracet in the rabbit. Diatrizoate sodium was found to produce earlier and better visualization of the upper urinary tract with less than half the systemic toxicity observed with iodopyracet.

6. The acute intravenous toxicity of diatrizoate sodium in the mouse, rat, rabbit, cat, and dog was found to be approximately half as great

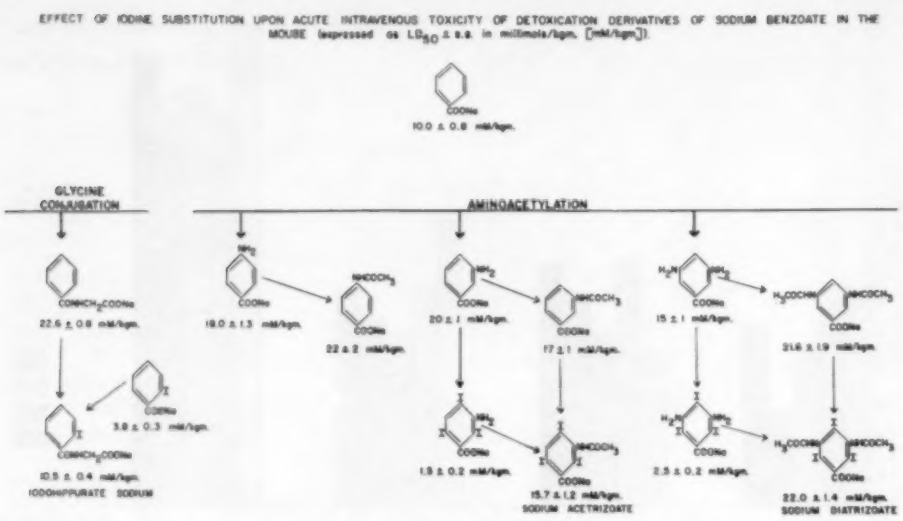

EFFECT OF IODINE SUBSTITUTION UPON ACUTE INTRAVENOUS TOXICITY OF DETOXICATION DERIVATIVES OF SODIUM BENZOATE IN THE MOUSE (expressed as $LD_{50} \pm$ S.E. in millimols/kgm. [mM/kgm.])
10.0 ± 0.8 mM/kgm.
GLYCINE CONJUGATION
AMINOACETYLATION
22.6 ± 0.9 mM/kgm.
3.8 ± 0.3 mM/kgm.
10.5 ± 0.4 mM/kgm.
IODOHIPPURATE SODIUM
19.0 ± 1.3 mM/kgm.
22 ± 2 mM/kgm.
20 ± 1 mM/kgm.
17 ± 1 mM/kgm.
1.9 ± 0.2 mM/kgm.
15.7 ± 1.2 mM/kgm.
SODIUM ACETRIZOATE
15 ± 1 mM/kgm.
21.6 ± 1.9 mM/kgm.
2.5 ± 0.2 mM/kgm.
22.0 ± 1.4 mM/kgm.
SODIUM DIATRIZOATE

Fig. 8.—The acute intravenous toxicity of iodohippurate sodium, sodium acetrizoate, and sodium diatrizoate, expressed in terms of mM/Kg. in the mouse, before and after the incorporation of iodine (78). (Reproduced by permission of The Williams & Wilkins Co.)

as that observed for iodopyracet, sodium iodomethamate, or sodium acetrizoate.

7. The incorporation of iodine into both sodium 3-acetamidobenzoate and sodium 3,5-diacetamidobenzoate had no effect on toxicity when measured in terms of mM/Kg. by intravenous injection in the mouse, indicating that radiopacity had been conferred upon these molecules with no expense in terms of systemic toxicity.

8. The urographic contrast medium also appears to consist of (*a*) an iodinated aromatic nucleus to provide radiopacity and (*b*) a carboxyl group or, at most, an alkanoic acid side chain with no more than three carbons to provide water solubility.

9. The use of both test procedures in the search among iodinated organic compounds for improved X-ray contrast media was illustrated by a description of the emergence of iopanoic acid and diatrizoate sodium from unknown chemical compounds in the laboratory to useful diagnostic aids to the radiologist in the form of individual medicinal entities known as Telepaque and Hypaque, respectively.

CONCLUSION

I would like to point out that in the search for a new medicinal agent, a research idea is much like a new-born infant; it demands a great deal of expert help from many sources. The process of creating a new medicine is complicated and requires the skills of many highly trained people functioning as a closely coordinated team. I have been extremely fortunate in having the opportunity to work with a most stimulating group of research investigators at the Sterling-Winthrop Research Institute. I am deeply grateful to Dr. M. L. Tainter, our Director of Research, whose encouragement and guidance has been a great source of help and inspiration to me. I am particularly grateful to Dr. Sydney Archer and Dr. Aubrey Larsen, whose chemical ingenuity has made these studies possible. I wish to thank the many members of the Chemistry and Biology Divisions who have helped in the successful completion of these studies. I would like to emphasize especially the importance of the contributions of the research staff of our Pharmacy Division, whose specialized technical training has helped solve the many problems involving compounding, solubility, stability, and formulation. I would also like to acknowledge, with grateful appreciation, the technical assistance of Mr. John Romano, Mr. Armand Brousseau, Mr. Leon Duprey, and Miss Nancy Harvey. Finally, I wish to express my sincere thanks to the Chilean Iodine Educational Bureau, Inc., for the honor of having been named recipient of their 1957 Iodine Research Award.

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A New Highly Reactive Aluminum Hydroxide Complex for Gastric Hyperacidity I*

In Vitro Rate of Acid Neutralization

By E. T. HINKEL, Jr., M. P. FISHER, and M. L. TAINTER

A new, highly reactive form of aluminum hydroxide polymer has been developed which takes up acid faster and in larger amounts than conventional aluminum hydroxide dried gel under conditions similar to those existing in the hyperacid stomach. Using this new form of aluminum hydroxide a tablet has been compounded which, in the recommended dose, neutralizes acid several times faster than comparable tablets in their therapeutic doses, at pH levels which will practically inactivate gastric pepsin. It also neutralizes about three times as much total acid as previously available tablets under these same conditions. The new tablet, as a result of this greater activity, should provide a more effective means of controlling gastric hyperacidity than previously available tablets.

LIQUID ALUMINUM HYDROXIDE preparations have long been recognized as being highly effective in the management of gastric hyperacidity. However, from the standpoint of convenience to the patient it is much more desirable to use a dry dosage form, such as a tablet. Unfortunately, the process of drying these gels for such use may destroy much of the original beneficial effect. Investigation in these laboratories of the factors controlling this loss of activity has resulted in the development of a new, especially reactive polymeric aluminum hydroxide complex. The purpose of this paper is to present the antacid properties of a tablet prepared from this material in comparison with existing products.

Prior to the introduction of aluminum hydroxide products, the *in vitro* evaluation of gastric antacids consisted essentially of determining their total acid consuming capacity. The major ingredients of these antacids, such as sodium bicarbonate, calcium carbonate, magnesium carbonate, and the like, reacted rapidly and completely with either dilute acid or gastric juice. However, dry aluminum hydroxide preparations differ widely in their antacid power in both rate and completeness of reaction, and are greatly influenced by the reaction conditions. As a result, the total acid neutralizing power of such preparations may be practically meaningless as an indication of therapeutic efficacy.

Many *in vitro* methods for the evaluation of antacid products have been utilized (1–5). With few exceptions these procedures are modifications of those given by Johnson and Duncan (6) and Rossett and Flexner (7), in which acid is added to a given amount of antacid. The pH *versus* time is recorded. The aim of many investigators has been the development of a testing method which uses the equivalent of an artificial stomach. Some use artificial gastric juice or devices to withdraw portions of the "stomach" contents, and the like. In theory these are appealing. However, the variables encountered clinically, even in the same patient, are so numerous (8) that they make improbable an *in vitro* duplication of the human stomach.

As far as can be determined, the method developed by Rossett, *et al.*, has given the best *in vitro-in vivo* correlation (7, 9, 10, 11). Their main contribution has been recognition of the fact that dilute acid or gastric juice has to be added to the antacid continuously at a sufficiently rapid rate to simulate maximal secretion rates. Even the more refractory and therapeutically unsatisfactory aluminum hydroxide products will show respectable "activities" if acid addition is slow enough. Their method, then, is useful in measuring speed of reaction and indicating ability to hold gastric pH within desired limits.

A different approach to the problem has been taken by Bachrach (12) and by Gwilt (15). Their method consists essentially of adding dilute acid to the antacid at such a rate as to maintain a pH of 3.5. This method gives a direct estimate of the speed of action and total available activity, whereas the previous procedures demonstrate primarily the buffering effect as measured by pH and duration of action.

Attention in this paper is directed to the fallacy of considering speed as the sole criterion of effectiveness. Were this the case, sodium bicarbonate and the alkaline-earth carbonates would not have fallen into disfavor. It is true that they react almost instantly with acid, but they buffer at too alkaline a pH where inhibition of peptic activity is complete and where acid rebound

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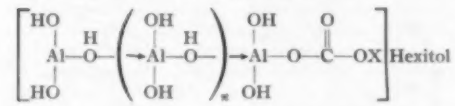
We are grateful to Mr. Joseph Westheimer, Mrs. Agnes Hamm, and Mr. J. W. Baum for their assistance during the prosecution of this project.

is likely. Therefore, it becomes necessary to establish, in addition to rate, the buffering range. A subsequent paper will deal with this aspect of the problem. However, the Bachrach method, as mentioned, is ideal for study of the rate and total activity and has proved very valuable in the development of a new, highly reactive aluminum hydroxide antacid tablet which is described in this paper.

EXPERIMENTAL

The materials studied were ten leading aluminum hydroxide antacid tablets. These contain aluminum hydroxide as their chief antacid ingredient, which in some is reinforced by addition of magnesium hydroxide, oxide, peroxide, or trisilicate, or calcium carbonate. They are identified in Table I as tablets 1–10. The new tablet, Creamalin tablets (Winthrop Laboratories), presented herein contains a new, highly reactive polymeric aluminum hydroxide hexitol complex in an amount equivalent to 320 mg. of aluminum hydroxide dried gel and 75 mg. of magnesium hydroxide. The structure of this polymer has not been completely elucidated. However, it can be represented by the partial formula



where n is at least 1 and averages less than 6, and X is a cation.

The procedure of Bachrach (12) was employed. Since many of the antacid tablets are intended to be chewed and do not disintegrate readily in aqueous media, all the tablets tested were ground in a mortar to pass a 60-mesh screen. Aliquots of the powdered antacid equalling the weight of an average tablet were suspended in 40 ml. of distilled water in a constant temperature (37°) cell equipped with a mechanical stirrer and pH electrodes. Ten drops of a 10% aqueous solution of a nonionic surfactant (Triton X-100) were added to insure wetting, since it was found that otherwise many products would not readily disperse. One-tenth normal hydrochloric acid was added continuously from a buret in just sufficient volumes to maintain the pH at 3.5. The volumes of acid added were recorded at frequent intervals for one hour. The end point, pH 3.5, is within the therapeutically desirable range, since at this level peptic activity is practically stopped, and the excessive acidity of the ulcer patient is reduced to nonirritating concentrations.

Since the products other than the Creamalin tablets were of indeterminate age when purchased, their stability was studied. There was no significant change in their activities after six months at room temperature, and, therefore, it was concluded that the results obtained initially represented a reliable measure of their usual activity.

Selection of Titrant. Although some investigators prefer to use an artificial gastric juice for their titrations, this study was carried out with 0.1 *N* HCl for two reasons. First, the presence of a protein can hinder the efficient operation of the glass electrode due to adsorption on the surface of the sensitive glass membrane (13). Second, the concentrations of enzyme, salts, etc., *in vivo* vary over too broad a range to permit the preparation of a truly representative artificial gastric juice.

Selection of Dosage.—There are a number of bases on which the various antacid tablets might be compared. These include tablet *vs.* tablet, by average label dose, by weight of active ingredients, or by equivalent total acid combining capacity. A tablet *vs.* tablet comparison has little meaning since the size

TABLE I.—PERTINENT DATA FOR THE ANTACID PRODUCTS EVALUATED

Product	Antacid Active Ingredients	Av. Tablet Wt., mg.	Label Dose, Tabs.	Av. Label Dose, Tabs.	Total Acid Consuming Capacity, ml. 0.1 *N* HCl per Tablet	Total Acid Consuming Capacity, ml. 0.1 *N* HCl per Gm. Active Ingredients	Number of Tablets to Neutralize 250 ml. 0.1 *N* HCl	Number of Tablets to Contain 1 Gm. Active Ingredients
New Creamalin	Aluminum hydroxide[a] 320 mg. Magnesium hydroxide 75 mg.	675	2–4	3.00	125	318	2.00	2.54
No. 1	Aluminum hydroxide[a] 400 mg. Magnesium hydroxide 100 mg.	913	1–2	1.50	112	224	2.24	2.00
No. 2	Aluminum hydroxide[a] 784 mg.	1055	0.5–1	0.75	225	288	1.11	1.28
No. 3	Aluminum hydroxide[a] 150 mg. Magnesium trisilicate 250 mg.	552	1–2	1.50	79	198	3.17	2.50
No. 4	Aluminum hydroxide[a] 250 mg. Magnesium trisilicate 500 mg.	1262	1–4	2.50	87	115	2.88	1.32
No. 5	Aluminum hydroxide[a] 400 mg. Magnesium oxide 200 mg.	795	1–2	1.50	170	283	1.47	1.66
No. 6	Aluminum hydroxide[a] } 400 mg. Magnesium hydroxide }	467	2–4	3.00	84	210	2.97	2.50
No. 7	Aluminum hydroxide[a] 648 mg. Magnesium trisilicate 324 mg.	1355	0.5–2	1.25	189	195	1.32	1.03
No. 8	Aluminum hydroxide[a] 144 mg. Calcium carbonate 71 mg. Magnesium peroxide 86 mg.	602	1–2	1.50	101	336	2.48	3.33
No. 9	Aluminum hydroxide[a] 97 mg. Magnesium trisilicate 292 mg.	398	3–5	4.00	60	151	4.16	2.52
No. 10	Aluminum hydroxide[a] 300 mg.	800	2–4	3.00	80	266	3.13	3.33

[a] Calculated as aluminum hydroxide dried gel, U. S. P.

of the tablet is arbitrarily selected by the manufacturer. The comparison is more meaningful when based on the average dose recommended for the relief of gastric hyperacidity. Therefore, the first comparison was made in the present study on a weight of ground tablet equivalent to the average label dose.

In addition, comparison of equal weights of the antacid ingredients provides a measure of the inherent efficiency of the antacids selected, independent of tablet size or dosage recommendations. Therefore, a weight of tablet material representing 1.0 Gm. of the combined antacid ingredients of each tablet was calculated, and this information was used to prepare a second series of titration curves.

The third useful basis for comparison is derived from the total acid neutralizing power of the tablet mix. This can be measured readily by a determination of the total acid consuming capacity using the extreme conditions of the U. S. P. procedure (14). From these values the weights of tablet material can be selected which will have the same total acid combining power, e. g., 250 ml. of 0.1*N* HCl, and the comparisons of speed, etc., made at these equivalent weights.

In practice it is more convenient to use aliquots of the powdered antacid preparation to avoid adding excessive volumes of acid in the titrations. However, it was determined experimentally that for any given powder there is a direct relationship between the weight of tablet material titrated and the volume of acid required. Thus a simple calculation is all that is necessary to convert the values obtained for any given weight of material to those applicable to another quantity.

RESULTS

In Table I are summarized pertinent measurements on the eleven tablets included in this study. They comprise ten of the leading aluminum hydroxide antacid tablets and the new Creamalin tablet.

The weight of the combined antacid ingredients in the tablets varied from 300 to 960 mg. The total size of the tablets also showed a wide range because of the presence of diluents, binders, and nonantacid ingredients. The smallest was 398 mg. and the largest, 1,355 mg. The total acid neutralizing capacity of the tablets, as determined by the U. S. P. method, also varied widely. Tablet No. 9 neutralized only 60 ml. of acid in this test while the greatest capacity was shown by tablet No. 2, which neutralized 225 ml. of acid.

There was also calculated the number of tablets required to neutralize 250 ml. of acid under these conditions. The table shows that there was more than a three-fold difference in this measure of activity. The size of the tablet obviously modifies the amount of acid it can neutralize. However, it can be seen from the table that when the quantities of tablets required to contain 1.0 Gm. of active ingredients are compared, there is still more than a three-fold difference between the extremes.

The data illustrate quite clearly that the innate reactivity of aluminum hydroxide and related antacids varies widely among different preparations and is influenced strongly by factors other than tablet size and composition. In this measurement of total antacid power, which does not disclose speed or buffering power, the new Creamalin tablet occupied an intermediate position when considered on a tablet basis, because the tablet is smaller than about half those considered. When the data are considered in terms of capacity per Gm. of active ingredients, the new Creamalin tablet will be seen to have considerably more antacid power than any of the other tablets of similar composition.

The most important test, however, of potential clinical value is not the assay of total activity as described by the U. S. P., but rather the speed with which acid can be taken up while maintaining a pH which will inhibit peptic activity. The results of titrations of these materials, carried out to a constant pH as described above, are shown in Figs. 1, 2, and 3.

In Fig. 1 the activities per Gm. of antacid ingredients are plotted. It will be noted that the new Creamalin tablet neutralized acid faster than nine of the other tablets. It also neutralized more acid in the sixty-minute titration period than the others by a very appreciable margin. This test more nearly approaches physiological conditions than the U. S. P.

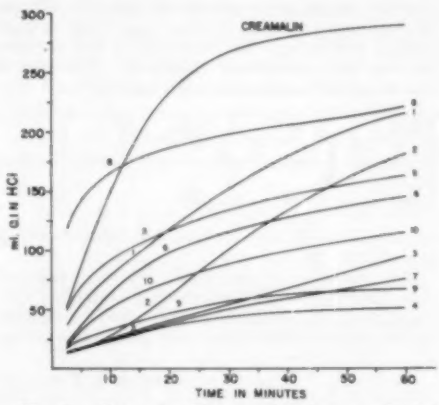


Fig. 1.—Rate of acid neutralization at pH 3.5 and 37°. Sample equals weight of tablet material containing 1.0 Gm. active ingredients.

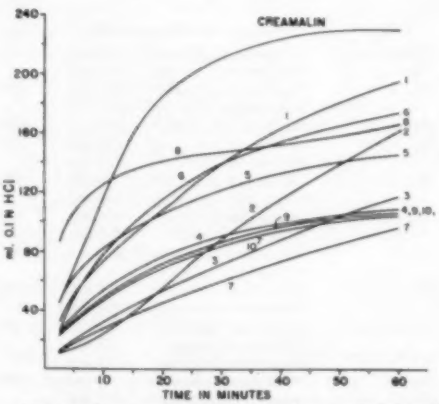


Fig. 2.—Rate of acid neutralization at pH 3.5 and 37°. Sample equals weight of tablet material that will neutralize 250 ml. 0.1 *N* HCl by U. S. P. method.

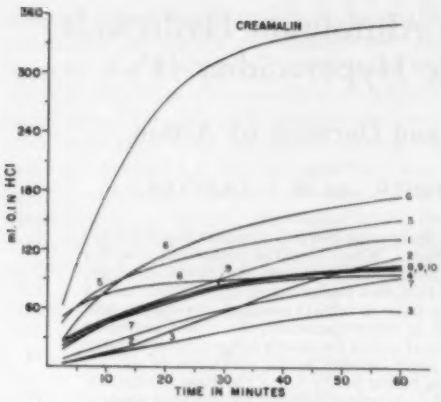


Fig. 3.—Rate of acid neutralization at pH 3.5 and 37°. Sample equals weight of tablet material representing average label dose.

procedure and hence has considerable significance in suggesting superior therapeutic activity for the new tablet.

It might be thought that this greater reactivity is the result of comparing the tablets on a weight rather than a chemically equivalent basis. Hence, in Fig. 2 are shown the values when the comparisons are made for quantities of the antacid which will neutralize 250 ml. of 0.1 *N* HCl under U. S. P. conditions. Again the superiority of the new Creamalin is manifest. By the end of thirty minutes, for example, the new Creamalin has exerted 85% of its theoretical antacid capacity. For the same time interval the next best was only up to 60%. For the forty-, fifty-, and sixty-minute intervals the ratios of Creamalin acid consumption (in per cent) to the next best were 90:65, 92:72, and 93:79, respectively.

In addition, the patient is interested in the extent and rapidity of his relief when taking the recommended dose. In Fig. 3 the values obtained using this very practical criterion are set forth. It will be noted immediately that when this usual clinical dosage is titrated, the new Creamalin was markedly superior. It reacted much more rapidly throughout the entire titration and ended up by neutralizing approximately three times as much as the other tablets. This suggests a decided therapeutic advantage at the recommended dosage in both onset of relief and in the total amount of acid neutralized, resulting in better control of gastric hyperacidity.

DISCUSSION

It is widely recognized that aluminum hydroxide dried gels from different sources vary considerably in their physical and chemical properties. The most important difference when considering them as antacids is, of course, reactivity with acid. It becomes extremely important, then, to define precisely what is meant by this reactivity. Albeit the mechanism of reaction between aluminum hydroxide and hydrochloric acid is rather poorly defined, it is quite easy to demonstrate experimentally certain variations depending on the conditions chosen.

When studying total acid consuming power, the classical assay methods utilize a large excess of hydrochloric acid to react with the antacid and back titrate to determine the amount consumed. As can be expected, the reaction is forcefully driven towards completion. In the light of the present therapeutic considerations the data thus obtained can be misleading. This is especially true in the case of those products whose rates of reaction change with time, for not only must a product neutralize the acid, but it must do so rapidly to achieve prompt relief. In other words, the rapidity of reaction is directly proportional to the slope of the titration curve. Of course, it is possible to effect a high initial velocity by including such ingredients as magnesium hydroxide, alkali bicarbonates, and alkaline-earth carbonates. Magnesium hydroxide has become quite popular for this purpose due to its attendant laxative properties. However, once these instantly reacting compounds are neutralized, the antacid effect becomes a function solely of the aluminum hydroxide present. The reactivity of the aluminum hydroxide, then, is the determining factor in the persistent efficacy of this type of antacid.

As mentioned, these differences are not readily apparent under the customary procedures of assay. Also the conditions prescribed do not represent the environment encountered by aluminum hydroxide in the stomach. Hence, when more physiological test conditions are set up, many of the antacid preparations are found to be much less effective than would be anticipated.

Guided by this new concept of therapeutic reactivity it has been possible to make a highly reactive complex aluminum hydroxide polymer which takes up acid quickly and in almost theoretical amounts under physiological conditions. From this has been formulated a new tablet which makes these new properties available therapeutically.

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A New Highly Reactive Aluminum Hydroxide Complex for Gastric Hyperacidity II*

In Vitro Buffering Range and Duration of Action

By E. T. HINKEL, Jr., M. P. FISHER, and M. L. TAINTER

A new, highly reactive polymeric aluminum hydroxide hexitol complex has been developed and incorporated into an antacid tablet. When tested in comparison with a liquid aluminum hydroxide gel of proved clinical effectiveness, the new Creamalin tablet has an almost identical duration of action and buffering range. When tested in comparison with ten leading aluminum hydroxide tablets using an *in vitro* method recognized as having good correlation with *in vivo* experience, the new Creamalin tablet exerts its buffering activity in the optimal range for much longer periods than the others. Effective antacid therapy has been defined as the elevation of gastric pH to between 3.0 and 5.0 with the optimum being 3.5 to 4.5. The new Creamalin tablet should provide a highly effective and convenient antacid for poising gastric hyperacidity within this optimal range.

A PREVIOUS REPORT (1) presented a study of the rate of acid neutralization of a new antacid tablet, Creamalin tablet (Winthrop Laboratories), in comparison with ten similar antacid tablets. The new Creamalin tablet contains a specially prepared, highly reactive polymeric aluminum hydroxide hexitol complex. It was shown to have a much greater rate of acid neutralization than the other tablets tested. The present study was undertaken to evaluate the same antacid tablets from the additional standpoints of buffering range and duration of action.

It is felt that no single *in vitro* test method can furnish all the desired information regarding speed, duration, and buffering. Most of the previous work on this problem has employed the procedures of Johnson and Duncan (2), Rossett and Flexner (3), and various modifications thereof. In these procedures, acid is added to a given amount of antacid under physiological conditions and pH *versus* time recorded. The main difference in these methods is the manner in which acid is added to the reaction mixture. Some investigators add a single, large dose of acid while others employ the intermittent addition of small amounts. The method of Rossett and Flexner (3), and its subsequent refinement by Rossett and Rice (4), is reported to give results correlating very well with those obtained clinically. The latter state that the addition of acid at the rate of 240 ml. of 0.1 *N* hydrochloric acid per hour most nearly simulates the rate of secretion of the hyperchlorhydric stomach.

In order to interpret the data obtained by these methods, it is necessary to define the buffering range which will provide the most desirable antacid effect. The main objective of antacid therapy is the establishment of a level of gastric acidity that will permit healing and relieve pain with the least interference with essential digestive requirements. It is impossible to assign an absolute value for the most effective pH for this purpose; however, it is possible to select an effective range. Various pH ranges, 3.5–4.0 (2), 4.0–5.0 (5), 4.0–5.5 (6), and 4.0–6.0 (7), have been proposed, none of which seems to be entirely satisfactory. It is agreed that reduction in the free acidity of the stomach relieves the discomfort due to gastric hyperacidity, and it has been reported that relief may be achieved even at pH 2.5 (3). This seems probable considering the decrease in absolute acidity. Raising the pH of gastric juice (pH 1.5) to pH 2.5 or pH 3.0 represents a decrease in hydrogen ion concentration of 90 or 97 per cent, respectively. However, it seems safer to set pH 3.0 as the lower limit of the desirable buffering range.

Healing of peptic ulcers is facilitated by a decrease in peptic digestive activity. Peptic digestion is maximal at about pH 2.0 and decreases rapidly with increasing pH. All peptic activity ceases long before the stomach contents become neutral in a chemical sense. In fact pepsin is only very slightly active at pH 4.0 and inactive at pH 5.0. Kirsner (8) states that healing probably occurs at a pH lower than 4.0, indicating that complete abolition of peptic activity is not necessary. In addition, it is well established that alkalinizing the stomach is undesirable due to the possibility of acid rebound and alkalosis. Therefore, it appears unnecessary to raise the pH above 5.0 to control gastric hyperacidity and to permit healing of ulcers.

In view of the above facts it is evident that the

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main objective of antacid therapy can be met by maintaining the pH of the stomach contents within the range of pH 3.0–5.0. Since gastric hyperacidity is being very successfully managed with liquid aluminum hydroxide preparations, and since these exert their maximum buffering between pH 3.5 and 4.5, this still narrower range can be considered the optimal. The efficacy of an antacid, then, is directly dependent upon its ability to maintain the gastric contents within the prescribed ranges for prolonged periods of time.

EXPERIMENTAL

The materials studied were those employed in our prior investigation (1), namely, the new Creamalin tablet and ten leading aluminum hydroxide antacid tablets of comparable composition. This Creamalin tablet contains the new, highly reactive polymeric aluminum hydroxide hexitol complex in an amount equivalent to 320 mg. of aluminum hydroxide dried gel and 75 mg. of magnesium hydroxide. The other tablets all contain aluminum hydroxide as a major ingredient, alone or in combination with other antacid ingredients. Their compositions are given in Table I of our previous report (1), along with other pertinent data.

The procedure of Rossett and Rice (4) was modified by conducting the titrations at 37° in the presence of a small amount of wetting agent. The tablets were prepared by grinding to pass a 60-mesh screen. Aliquots of the powdered tablets were added to 70 ml. of distilled water in a constant temperature (37°) cell equipped with a mechanical stirrer and pH electrodes. The pH was tracked by a continuous electronic recorder. The pH electrode assembly in the cell consisted of a glass electrode and a salt bridge connecting to an outside calomel electrode according to the technique of Duggan and Stevens (9). Preliminary experiments showed that the calomel electrode became contaminated when placed in the reaction mixture.

Ten drops of a 10% nonionic surfactant (Triton-X-100) solution were added to insure wetting and dispersion since some tablets contain lubricants and other hydrophobic materials. At time zero, 30 ml. of 0.1 *N* HCl were added to the suspension, giving the equivalent of 100 ml. of 0.03 *N* HCl. Continuous addition of 0.1 *N* HCl was started immediately at the rate of 240 ml. per hour. The resulting pH changes were continuously plotted by the recorder. Triplicate runs were made, and the results averaged.

The dosages used for comparison were the same as those employed in our previous report (1). They were amounts of powdered tablets containing, (*a*) the average label dose, (*b*) 1.0 Gm. of active antacid ingredients, or (*c*) a total acid-consuming capacity (U. S. P.) (10) of 250 ml. of 0.1 *N* HCl.

To confirm the desirable buffering range, titrations were carried out similarly on a liquid aluminum hydroxide gel of proved clinical effectiveness. Since the liquid had a total acid-neutralizing capacity (U. S. P.) (10) of 21.7 ml. of 0.1 *N* HCl per Gm., 11.52-Gm. samples (providing 250 ml. of 0.1 *N* HCl total acid-neutralizing capacity) were included in the third comparison.

RESULTS

The titration curves of the products are given according to the three dosage comparisons described above. The titrations for most products were continued until the pH value dropped to pH 2.25 or below, at which point the antacid capacity can be considered practically exhausted. Since several products failed to raise the pH to this value, or exceeded it for only a short time, all titrations were continued for at least thirty minutes to show their general shape. The speed of pH change, buffering range maintained, and duration of antacid effect can be read from the curves presented in the figures.

Figures 1*a* and 1*b* show the results obtained for the tablets and the liquid aluminum hydroxide gel when the comparison was made on the equivalent acid-neutralizing capacity basis. Note that the

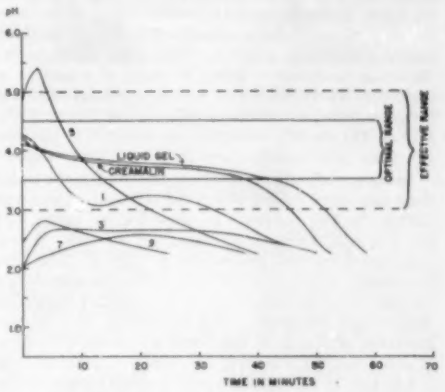

Fig. 1a.—Duration of action and buffering range at 37°. Samples equal weight of tablet material that will neutralize 250 ml. 0.1 *N* HCl by U. S. P. method. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

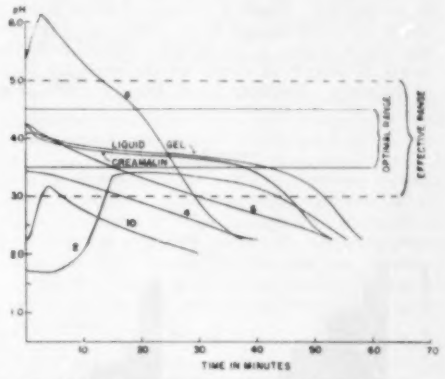

Fig. 1b.—Duration of action and buffering range at 37°. Samples equal weight of tablet material that will neutralize 250 ml. 0.1 *N* HCl by U. S. P. method. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

liquid gel does indeed buffer within the optimal range, pH 3.5–4.5, and that approximately 85% of its effective duration of action is exerted therein. The curves for the new Creamalin tablet and the liquid are practically superimposable for their duration in the optimal range. This is due to the fact that the usual adverse effects of the drying and tableting operations on the activity of aluminum hydroxide gel have been largely eliminated in the new tablet. In contrast, only two other products, No. 1 (Fig. 1*a*) and No. 2 (Fig. 1*b*), exhibited any buffering in the broad effective range, pH 3.0–5.0. Three products, Nos. 3, 7, and 9 (Fig. 1*a*), showed buffering capacity but only at a level below the minimum effective limit. The others failed to show any significant buffering at all. It is also interesting to note the general shape of the curves for products Nos. 1 and 2. No. 1 shows an initial rapid fall for about the first ten minutes and then begins to exert its main buffering action. With No. 2 there is a latent period of fifteen minutes before this product begins its antacid activity. This would seem to indicate an undesirable delay in onset of action in a condition where rapid action is an essential requisite for prompt relief. Products No. 5 (Fig. 1*a*) and No. 8 (Fig. 1*b*) rapidly raise the pH above 5.0, but the pH then falls rapidly through the desired ranges.

In order to further quantitate the results, the times were measured during which the pH was maintained within the two ranges, i e., the effective range, pH 3.0–5.0, and the optimal range, pH 3.5–4.5. These data are presented graphically in Fig. 2. The new Creamalin tablet held the pH in the broader range for forty-five minutes and in the optimal range for thirty-eight minutes. This represents 85% of the duration of action of the liquid gel in these ranges. It also has approximately $1^1/_2$ and $2^1/_2$ times as long a duration in the effective and optimal ranges as the next best tablets. Six of the tablets tested never brought the pH into the optimal range, and three of these did not even reach the lower effective limit.

The values obtained when using the average label dose as the basis of comparison are presented in Figs. 3*a* and 3*b*. It is quite evident from inspection that the new Creamalin tablet surpasses all of the others in its duration of action. The only other product which maintains the pH in the prescribed ranges for any length of time is No. 6, but its actual buffering power is meager as shown by the declining slope of the curve. Products No. 5 (Fig. 3*a*) and 8 (Fig. 3*b*) drop quickly through these ranges after an initial rise to the upper limit. Product No. 2 (Fig. 3*b*) again shows a prolonged onset of action and reaches pH 3.0 for a very short interval. All the others buffer below the minimum acceptable level of pH 3.0.

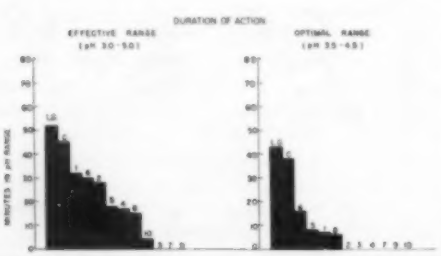

Fig. 2.—Duration of action in effective and optimal ranges. Samples equal weight of tablet material that will neutralize 250 ml. 0.1 *N* HCl by U. S. P. method. L.G.—liquid gel. C—Creamalin.

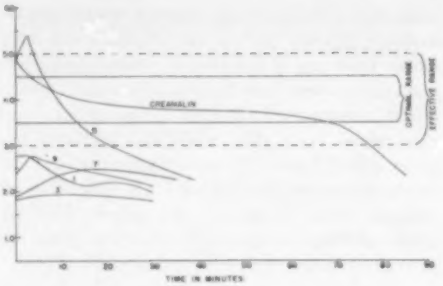

Fig. 3a.—Duration of action and buffering range at 37°. Samples equal weight of tablet material containing average label dose. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

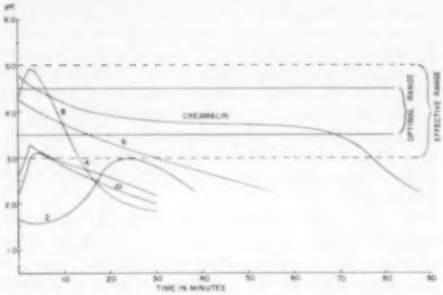

Fig. 3b.—Duration of action and buffering range at 37°. Samples equal weight of tablet material containing average label dose. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

The durations of action within the specified ranges for the average label doses are summarized in Fig. 4. The Creamalin tablet was active for sixty-five minutes in the optimal range and seventy-seven minutes in the effective range. This is 4 times and $2^1/_2$ times the duration of the next best product in the respective ranges. Seven products never produced any effect in the optimal range, and three did not even bring the pH into the broader effective range. An important observation from this test is that the recommended average dosage is probably insufficient to achieve a satisfactory gastric environment in a majority of the products tested.

The comparison based on quantities of the antacid tablets which would contain 1.0 Gm. of active ingredients is presented in Figs. 5*a* and 5*b*. As with Figs. 1 and 3, the same general characteristics are apparent. Once more it can be seen that the Creamalin tablet produces the most prolonged effect. Product No. 2 (Fig. 5*b*) appears as second best; however, it again shows a delay of about fifteen minutes before pH 3.0 is reached. Products No. 5 (Fig. 5*a*) and 8 (Fig. 5*b*) raise the pH undesirably

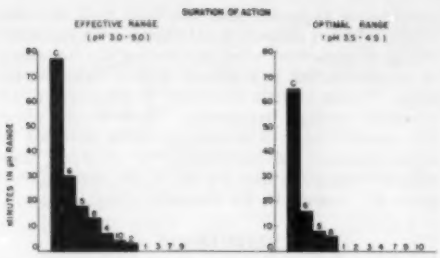

Fig. 4.—Duration of action in effective and optimal ranges. Samples equal weight of tablet material containing average label dose. C—Creamalin.

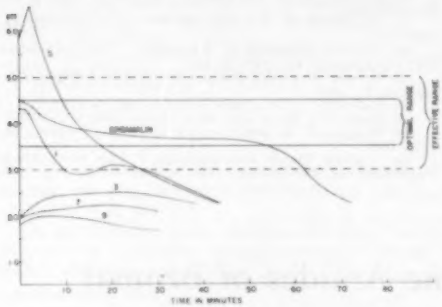

Fig. 5a.—Duration of action and buffering range at 37°. Samples equal weight of tablet material containing 1.0 Gm. antacid ingredients. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

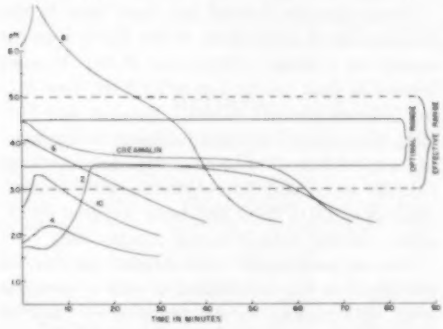

Fig. 5b.—Duration of action and buffering range at 37°. Samples equal weight of tablet material containing 1.0 Gm. antacid ingredients. Start with 70 ml. water plus 30 ml. 0.1 *N* HCl and add 0.1 *N* HCl continuously at the rate of 240 ml. per hour.

high at the start and do not show any prolonged buffering in the desired region. The curve for product No. 1 (Fig. 5*a*) also shows a characteristic dip to a point below pH 3.0 and only a short interval above this value. Product No. 6 (Fig. 5*b*) shows a steady decline indicative of limited buffering capacity. Product No. 10 held the pH above 3.0 for only seven minutes, whereas the remaining four products never reached this value.

The durations of action for this comparison are summarized in Fig. 6. The new Creamalin tablet maintains the pH within the optimal range for fifty-three minutes and within the effective range for sixty-two minutes. This is $2^1/_2$ and $1^1/_2$ times the duration, respectively, of the next best, product No. 2. Most of the other products show even less activity than can be accounted for by differences in the expected reactivity of their individual antacid ingredients.

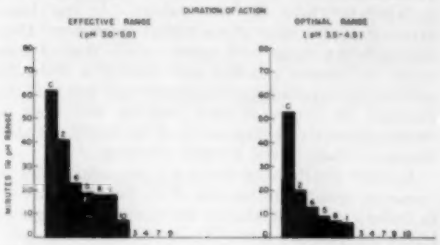

Fig. 6.—Duration of action in effective and optimal ranges. Samples equal weight of tablet material containing 1.0 Gm. antacid ingredients. C—Creamalin.

DISCUSSION

In the treatment of gastric hyperacidity the first concern of the patient is, of course, relief of discomfort. To be effective the antacid must have a rapid onset of action, i. e., it must reduce the acidity of the gastric contents quickly to a pH range, pH 3.0 to 5.0 and preferably pH 3.5 to 4.5, which will give the desired relief. Although many alkalies will quickly reach this range, most of them overshoot and raise the pH too high. An ideal antacid should rapidly bring the pH to within these limits without exceeding pH 5.0, and preferably buffering between pH 3.5 and pH 4.5. The success of liquid aluminum hydroxide gel is probably due to the fact that it exerts its action in this manner. However, it is well known that some of the previously available aluminum hydroxide tablets have given disappointing results. The difference in reactivity between these tablets and the gel is considered to be of serious clinical importance (4). It is in order then to review briefly some of the factors responsible.

Contrary to the popular conception, so-called aluminum hydroxide has a very complex chemical composition and exists in polymeric forms with varying states of hydration. Aluminum hydroxide that is readily reactive with acid at body temperature represents a very delicate variation of this general structure. This is especially true with respect to the extent of hydration. Depending upon the nature of the original gel and the manner in which it is dried, it is possible to make dried gels which react very avidly with acid or are almost completely inert.

Another factor which is very important is the technique of manufacture of the tablet. Most tablets contain, in addition to the active ingredients, an assortment of diluents, binders, lubricants, disintegrants, and other materials requisite to the preparation of an elegant tablet. Variations in disintegration times and the presence of hydrophobic materials definitely affect the availability of the ac-

tive ingredients, even in chewing tablets. In compression of the tablet, the pressure exerted within the die, in the order of five tons, may create enough heat to further dehydrate the polymer and contribute to its loss of activity. The pressure may also make the tablet so hard that it becomes difficult to disperse and chew.

Most of these difficulties have been overcome with the development of the highly reactive polymeric aluminum hydroxide hexitol complex which has been incorporated into the new tablet. It has been demonstrated under physiological conditions that the buffering range and speed of reaction of this tablet are almost identical with that of a clinically effective liquid aluminum hydroxide gel and that its duration of action is only slightly less than a chemically equivalent quantity of the liquid. It also disperses readily even without chewing.

Another attribute of the new Creamalin tablet is apparent from an inspection of its titration curves. It buffers in almost exactly the same range regardless of the quantity used. An increase in dosage prolongs the duration of action without significantly shifting the resulting pH zone, illustrating the desirable balance between aluminum hydroxide and magnesium hydroxide that has been built into the tablet. This resistance to pH change with increased dosage is important when considering the frequency of administration to a patient with a highly active ulcer. There is little likelihood of alkalinizing the stomach through overdosing. This should allow the establishment of dosage schedules designed to overcompensate for variable gastric emptying time with the assurance that the pH of the stomach contents will remain in the desirable range.

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The Preparation of Some Amides of Bromal*

By WILLIAM D. EASTERLY, Jr., and VAN R. ROSA

A series of amides, including both aliphatic and aromatic compounds, were prepared for the purpose of evaluating their toxicity, antifungal activity, antispasmodic activity, and sedative-hypnotic effect.

A REVIEW of the work with various amide derivatives indicates that very little study has been made of the bromal amides. Therefore, in extension of the study of substituted amides (1), a number of amide derivatives of bromal have been prepared. Some of these are new compounds; others have been synthesized previously.

Bischoff reported the preparation of bromalurethane in 1874 (2), and Schiff and Tassinari prepared bromalacetamide about the same time (3). In 1932 the condensation of bromal hydrate with urea (4) was reported.

Yelburgi (5), in 1933, reported the chemical properties of a number of bromal amides, prepared by condensing bromal hydrate with a series of aliphatic amides from formamide to pelargonamide. Meldrum and Deohar (6) extended this work by condensing bromal hydrate with aromatic amides, including benzamide, phenylacetamide, and salicylamide. Chattaway and James (7) prepared the methylurea derivative of bromal.

More recently, bromal has been used in the preparation of compounds of the DDT type for insecticide studies. Cristol and Haller (8) used bromal in their production of 1,1,1-tribromo-2,2-bis (*p*-bromophenyl) ethane. Balaban and Sutcliffe (9) prepared aryl trihaloethanes by condensing two different benzene compounds such as chlorobenzene and fluorobenzene with bromal. Analogs of DDT have also been prepared by reacting bromal with aromatic ethers (10).

The preparation of some bromal amides for investigation was undertaken in view of previous work with various substituted amides. Byrum and LaRocca (11) showed the potentialities of amide derivatives as sedative-hypnotics in their investigation of certain chloral and α,α,β-tributyraldehyde amides. Anticonvulsant activity has been demonstrated for certain amides of dichloroacetaldehyde by Easterly and LaRocca (1). Other substituted amides, the α-bromoacetamides, have revealed remarkable levels of fungus inhibition (12).

EXPERIMENTAL

General.—As no record has been found to date of their previous preparation, the phenylbutyramide

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Thanks are extended to Dr. J. P. LaRocca of the University of Georgia, School of Pharmacy, and to Dr. R. O. Bachmann of the University of Arkansas, School of Pharmacy, for their helpful suggestions.

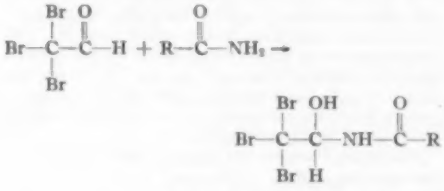
TABLE I.—BROMAL AMIDES[a]
$RCONHCH(OH)CBr_3$

Compound[b]	Yield, %	M. P. °C.[c]	Formula	Halogen Calcd. %	Halogen Found[d] %
Acetamide[e]	29	166	$C_4H_6Br_3NO_2$	70.8	72.2
α-Chloroacetamide	51	135	$C_4H_5Br_3ClNO_2$	73.5	72.1[f]
α-Phenylacetamide[g]	49	147	$C_{10}H_{10}Br_3NO_2$	57.7	56.2
Propionamide[h]	26	167[i]	$C_5H_8Br_3NO_2$	67.8	66.4
Butyramide[g]	30	146	$C_6H_{10}Br_3NO_2$	65.2	66.2
Isobutyramide[h]	33	154	$C_6H_{10}Br_3NO_2$	65.2	64.1
α-Phenylbutyramide	38	139	$C_{12}H_{14}Br_3NO_2$	54.1	53.5
Benzamide[g]	25	148	$C_9H_8Br_3NO_2$	59.7	58.8
Nicotinamide[j]	23	158	$C_8H_7Br_3N_2O_2$	59.6	59.6

[a] The "Chemical Abstracts" name for these compounds, N-(2,-2,-2-tribromo-1-hydroxyethyl) amides, was not used in this paper because it is unwieldy.
[b] All chemicals used were Eastman (Reagent) grade.
[c] Melting points taken on Fisher-Johns apparatus.
[d] Average of two determinations.
[e] Prepared previously by Schiff and Tassinari (3).
[f] The compound contains both chlorine and bromine, and the analysis is for total halogen.
[g] Prepared previously by Meldrum and Deohar (6).
[h] Prepared previously by Yelburgi (5).
[i] Melting point is lower than the 174° reported by Yelburgi (5).
[j] Reference (13).

and the chloroacetamide derivatives are reported as new; the preparation of the others has been reported (3, 5, 6, 13).

All the bromal amides reported in Table I were prepared by condensing bromal (tribromoacetaldehyde) with the appropriate amide. The general reaction equation is:



METHOD

Equimolar portions of bromal (tribromoacetaldehyde) and the appropriate amide were allowed to react, temperatures being maintained in a constant temperature bath. The optimum reacting temperature for the compounds prepared ranged from 21 to 37°. Condensation, indicated by the formation of a paraffin-like solid, varied from a few minutes for bromal nicotinamide to five days for bromal α-chloroacetamide. (The most suitable temperature and reacting time for the majority of the condensations was approximately twenty-four hours at 25°.)

The condensed masses were dissolved in hot alcohol, followed by purification and crystallization by essentially the same procedure as that previously reported for dichloroacetaldehyde derivatives (1).

Analytical Methods.—Each compound was first tested qualitatively for nitrogen and bromine by application of the sodium fusion process. Then quantitative determinations were made using the peroxide bomb fusion method for bromine.

As a check on procedure, a duplicate analysis for the bromine content of bromal acetamide was made by Oakwold Laboratories of Alexandria, Va.

The analytical data for the nine compounds are given in Table I.

SUMMARY

1. A satisfactory laboratory procedure for synthesizing some amide derivatives of bromal is described.

2. The physical properties of nine bromal amides are given.

3. Antifungal activity studies on the compounds have been conducted, with results to be published in another paper.

4. Additional pharmacological screening of the compounds is being carried out by The Upjohn Company, Kalamazoo, Mich.

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The Kinetics of Degradation of Chlorobutanol*

By A. DAMODARAN NAIR† and JOHN L. LACH

The degradation of chlorobutanol in aqueous solutions appears to be a specific hydroxide-ion-catalyzed reaction. The overall rate equation was found to be equal to k_1 [chlorobutanol] + k_2 [chlorobutanol] [OH^-]. The energy of activation and half-life periods were calculated. The half-life for the reaction in a buffered solution at pH 3 was calculated to be 90 years at 25° whereas the half-life under similar conditions at pH 7.5 was found to be 0.23 years.

CHLOROBUTANOL, a widely used bacteriostatic agent in pharmaceuticals, has been observed to undergo decomposition in solution. Ray and Basu (1) made solutions of chlorobutanol with and without sodium chloride and adrenaline hydrochloride, and determined the amount of chlorobutanol decomposed after autoclaving each solution in a cotton-plugged flask at 15 pounds pressure for thirty minutes. In addition to its loss due to volatilization, they observed that it is hydrolyzed to a considerable degree in solution after heating or on prolonged storage. van Esveld (2) stated that the adrenaline-chlorobutanol mixtures undergo no loss of potency even after storage for three months in ampuls. Gershenfeld (3) used chlorobutanol in dilutions of 1:200 to 1:1,000, and observed the antibacterial property against various organisms before and after heating the solutions for half-hour periods at 121, 100, and 65°. The results of his study showed that when heated, all solutions failed to show bacteriostatic properties against test organisms and spores used because of the decomposition of the preservative. He also observed that chlorobutanol decomposes readily in alkaline solution. Taub and Luckey (4) studied the effect of sterilization temperature, storage, and pH on the stability of solutions of chlorobutanol in ampuls. They noticed a drop in pH of the solution if the initial pH was above 4. They also observed that the decomposition is minimum between pH 3 to 6 in buffered solutions and between pH 3 and 4 in unbuffered solutions. Murphy, *et al.* (5), estimated the percentage of hydrolysis of aqueous chlorobutanol solutions at different hydrogen ion concentration after autoclaving at 121° for periods of time ranging from five to twenty minutes and states that the hydrolysis of chlorobutanol is slight at pH values below 5, but increases sharply in less acidic solutions. They also point out that when neutral or alkaline solutions of sufficiently high buffering capacity containing chlorobutanol are autoclaved the antibacterial effect is definitely impaired. Since the hydrolysis of chlorobutanol lowers the pH of unbuffered solutions the hydrolysis is therefore self-limiting.

Although a number of studies have been conducted on the stability of chlorobutanol in aqueous solutions, no attempt has been made quantitatively to study its decomposition. The purpose of this work, therefore, was to study the kinetics of degradation of chlorobutanol in aqueous solutions.

EXPERIMENTAL

Apparatus and Reagents.—Phosphoric acid buffers (0.4 *M*) of pH 2, 3, and 4; 0.4 *M* acetate buffers of pH 5 and 5.5; 0.4 *M* Phosphate buffers of pH 5.5, 6, 6.5, 7, and 7.5; constant temperature bath with Sargent heater, circulator, and regulator; thermometer calibrated to 0.1; Beckman pH meter; 5-cc. ampuls; and apparatus and reagents used in "The Determination of Chlorobutanol in Pharmaceuticals by Amperometric Titration" (6), with the exception that 0.025 *N* NaCl solution and 0.02 *N* alcoholic $AgNO_3$ solution were used in the place of 0.08 *N* solutions of NaCl and $AgNO_3$.

Procedure Used for the Determination of pH and Temperature Effect.—One-tenth gram of chlorobutanol was accurately weighed into 250-ml. volumetric flasks and solutions ranging in pH from 2 to 7.5 (in increments of 1 pH unit from pH 2 to 5 and of 0.5 unit from pH 5 to 7.5) were made by means of 0.4 *M* buffer solutions. About 3 cc. of these solutions were transferred to 5-cc. ampuls, sealed, placed in wire baskets, and immersed in constant temperature bath containing liquid paraffin. After a lapse of about three minutes to allow the solutions to equilibrate thermally, one ampul was taken out, chilled in cold water, and analyzed for ionic chloride content, as described in "The Determination of Chlorobutanol in Pharmaceuticals by Amperometric Titration" (6). This was repeated at definite intervals of time over a period of forty hours for solutions of pH 2 to 5.5 and for a shorter period of time for solutions of pH 6 to 7.5.

For solutions of pH below 6.5, measurements were made by setting the temperature of the bath at 93.5° and 85° and for solutions of pH above 6.5, measurements were made by setting the temperature of the bath at 65 and 75°. For solutions of pH 6.5, readings were taken at all the temperature settings mentioned above.

Initially, duplicate samples were withdrawn from the bath and analyzed, but this was later discontinued as a result of the degree of precision attained.

The equivalents of chloride ion found per liter of

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Abstracted in part from a dissertation presented by A. D. Nair to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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the chlorobutanol solution were calculated in the following manner:

$$\text{Equivalents of } Cl^-/\text{liter} = (\text{ml. } AgNO_3)/2$$

where 2 is the volume of the sample withdrawn for analysis. Since 3 Cl^- are liberated for every mole of chlorobutanol according to the stoichiometric equation

$$(CH_3)_2\text{—C(OH)—}CCl_3 \rightarrow 3\ Cl^-$$

the amount of chlorobutanol decomposed per liter of the solution can be calculated as follows:

$$\text{chlorobutanol moles/liter} = (\text{Eq. } Cl^-/\text{liter})/3$$

Procedure for the Determination of the Order of the Reaction with Respect to Chlorobutanol by Changing Its Initial Concentration.—Essentially the same procedure as above was carried out except for the necessary changes in the concentration of chlorobutanol. The temperature was held at 75° and the pH was maintained at 6.5. The other concentrations of chlorobutanol solutions used were 0.08 and 0.12%.

Procedure for Determining the Effect of Change of Molarity of Buffer Solutions and also the Effect of Change of Ionic Species of Buffer Solutions.—Three-tenths molar and 0.2 *M* buffer solutions of pH 6.5 were made and the above procedure was repeated. To note the effect of change of ionic species, 0.4 *M* phosphate buffer and 0.4 *M* acetate buffer, both of pH 5.5, were made and the rate of decomposition of chlorobutanol was studied.

Characterization of the Degradation Products.—Chlorobutanol (0.25 mole) was decomposed with 1 mole of NaOH dissolved in 800 cc. of distilled water (to obtain a 5% solution) at 5 to 10°. Volatile components were removed by means of a steam bath. The residual solution was acidified with phosphoric acid and steam-distilled, and 1,600 cc. of distillate was collected.

The above procedure was repeated using 5% alcoholic NaOH solution (the alcohol content being 90%). After removing alcohol and other volatile components on a steam bath, 800 cc. of water was added to the residue and steam-distilled as before, and 1,600 cc. of distillate was collected.

RESULTS AND DISCUSSIONS

The results obtained indicate that the degradation of chlorobutanol in aqueous solutions is a base catalyzed reaction. Since there is no change in *k*, the specific reaction constant, when the reaction was carried out in buffer solutions of different molarity and of different ionic species, the hydrolysis appears to be a specific hydroxide-ion-catalyzed reaction (Figs. 1, 2).

The rate equation for the degradation may be written as follows:

$$\text{Rate} = k\ C_{\text{chlorobutanol}}{}^{m}\ C_{OH}{}^{-n}$$

where *k* is the specific reaction constant. Since the study was conducted in buffer solutions, OH^- concentration remained constant during each run and the rate equation is reduced to:

$$\text{Rate} = k'\ C_{\text{chlorobutanol}}{}^{m}$$

where $k' = K\ C_{OH}{}^{-n}$.

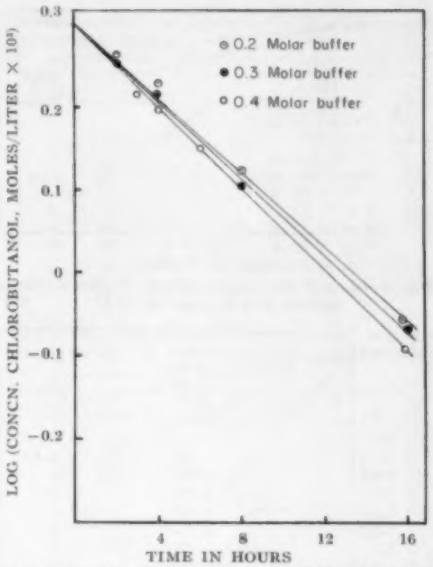


Fig. 1.—The effect of change of molarity of buffer on the rate of degradation of chlorobutanol at pH 6.5 and at 75°.

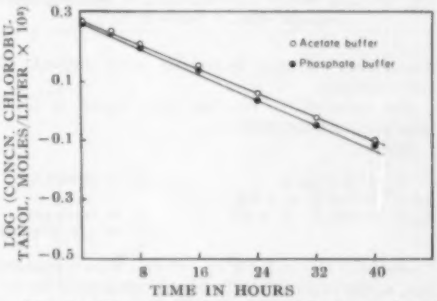


Fig. 2.—The effect of change of ionic species of buffers on the rate of degradation of chlorobutanol at pH 5.5 and at 85°.

The degradation is first order with respect to chlorobutanol, since a straight line relationship was obtained when the logarithm of the concentration of chlorobutanol was plotted against time at various pH, Figs. 3–6. This can be further verified, Fig. 7, by plotting the amount of Cl^- produced during the hydrolysis against the various initial concentrations of chlorobutanol, since chlorobutanol when decomposed liberates chloride ions according to the stoichiometric equation:

$$(CH_3)_2\text{—C(OH)—}CCl_3 \rightarrow 3\ Cl^-$$

In Fig. 8, the concentrations of Cl^- found after being held at 75° for sixteen hours at pH 6.5 have been plotted against the original concentrations of chlorobutanol.

It is obvious from the plot that the amount of Cl^- liberated is directly proportional to the initial concentration of the substrate, indicating the first order

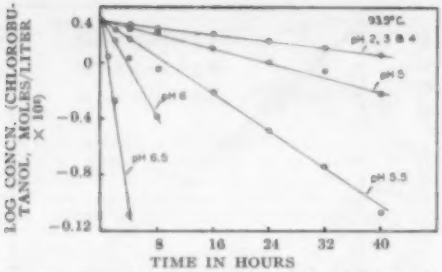


Fig. 3.—Log of the concentration of chlorobutanol against time in hours at 93.5°.

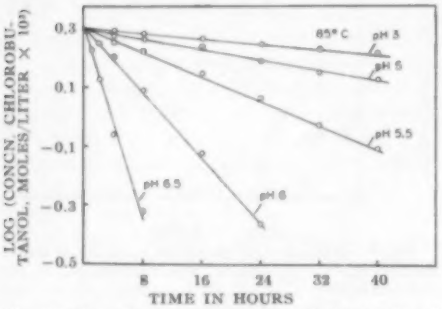


Fig. 4.—Log of the concentration of chlorobutanol against time in hours at 85°.

dependency of the hydrolysis with respect to chlorobutanol.

The order of the reaction with respect to OH^- may be obtained as follows:

Since

$$k' = K\,C_{OH^-}{}^{n} \qquad K = \text{constant}$$

$$\log k' = \log K + n \log C_{OH^-}$$

$$\log k' = \log K' + n\,pH \qquad K' = \text{constant}$$

$$pKw = pH + pOH$$

Therefore a plot of log k' *vs.* pH should be a straight line, n, the order of the reaction, being equal to the slope of the line. Figure 9 represents the plot of log k' *vs.* pH at 93.5 and 85°. This plot shows that the reaction, with respect to OH^-, is zero order from pH 2 to 4 and is first order from pH 5.5 to 7.5.

The overall rate may then be written as follows:

$$\text{rate} = \underbrace{k_1\,C_{(\text{chlorobutanol})}}_{1} + \underbrace{k_2\,C_{(\text{chlorobutanol})}\,(OH^-)}_{2}$$

Since the concentration of OH^- is very small at low pH, the second factor in the above rate equation becomes very small compared to the first factor and is negligible. Therefore, between pH 2 and 4, the rate is dependent on the concentration of chlorobutanol only, as indicated by the plot. From pH 5.5 to 7.5, the first factor is small and negligible when compared to the second factor in the above rate equation, and consequently the rate becomes dependent on the concentration of chlorobutanol as well as that of OH^-. Therefore, from pH 5.5 to 7.5, the rate is first order with respect to chlorobutanol and also first order with respect to OH^-, the overall reaction order being second order. Although this study was conducted up to pH 7.5, it

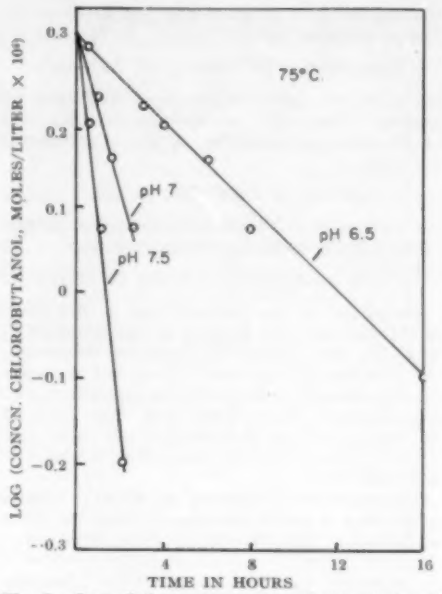


Fig. 5.—Log of the concentration of chlorobutanol against time in hours at 75°.

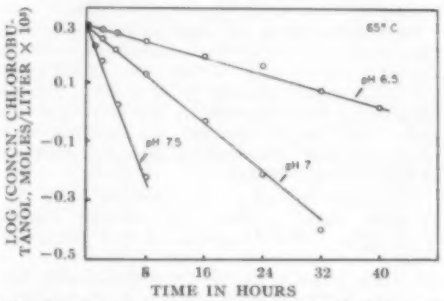


Fig. 6.—Log of the concentration of chlorobutanol against time in hours at 65°.

would follow that the first order dependency on chlorobutanol would hold true for pH above 7.5. Between pH 4 and 5.5, the order of the overall reaction is not strictly first order or second order.

Specific reaction constants for the degradation of chlorobutanol in aqueous solutions at various pH were determined at four temperatures (Table I) and the Arrhenius type plot of log k' *vs.* $1/T$ was observed to give a straight line (Fig. 10). The apparent energy of activation, E_a, for these reactions at various pH's was determined from the slopes and found to be constant at 30.7 ± 0.8 kilocalories. Since this apparent energy of activation includes the heat of ionization of water (approximately 12 kilocalories), the energy of activation for the hydroxyl reaction would be 18.7 kilocalories, a reasonable value for hydrolytic reactions of this type.

The half-lives for chlorobutanol in aqueous solution at 25° were determined from the calculated

TABLE I.—THE SPECIFIC REACTION CONSTANT, k', FOR THE DEGRADATION OF CHLOROBUTANOL AT DIFFERENT pH AND AT DIFFERENT TEMPERATURES

	Temp., °C.			
pH	65	75	85	93.5
	k', specific reaction constant, sec^{-1}			
2				3.95×10^{-4}
3	...	...	1.4×10^{-4}	4.06×10^{-4}
4	...	...	...	3.90×10^{-4}
5	...	...	2.86×10^{-5}	8.20×10^{-5}
5.5	...	...	6.74×10^{-5}	2.05×10^{-4}
6.0	...	...	1.81×10^{-4}	5.42×10^{-4}
6.5	4.43×10^{-5}	1.54×10^{-4}	5.98×10^{-4}	1.71×10^{-3}
7.0	1.36×10^{-4}	4.74×10^{-4}	1.66×10^{-3}[a]	4.42×10^{-3}[a]
7.5	4.17×10^{-4}	1.56×10^{-3}	5.29×10^{-3}[a]	1.42×10^{-2}[a]

[a] Calculated values from those experimentally obtained at 65 and 75°.

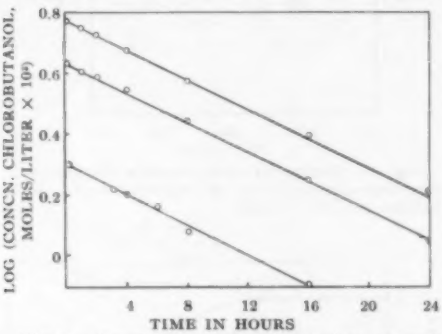


Fig. 7.—Log of the concentration of chlorobutanol against time in hours at pH 6.5 and at 75°. The plot shows the first order dependence of the reaction with respect to chlorobutanol, the rate of the reaction being directly proportional to the concentration of the substrate.

specific reaction constant, k' for reactions at various pH and at 25° (Table II, Fig. 11).

The Degradation Products.—The degradation products were qualitatively identified as acetone, carbon monoxide, chloride ion, and alpha hydroxyisobutyric acid. 2,4-Dinitrophenylhydrazine and *p*-nitrophenylhydrazine derivatives of acetone were prepared and the melting points were observed to be 126 and 152°, respectively. The presence of CO was detected by means of Saf-Co-meter[1] (carbon monoxide indicator). The chloride ion was detected with silver nitrate. The *p*-toluide and the *p*-nitrobenzyl ester of alpha hydroxyisobutyric acid were prepared and the melting points were observed to be 135–136° and 84–86°, respectively (which were 2 and 4° higher, respectively, from the reported ones). The amount of this acid obtained was very small in aqueous solutions: when chlorobutanol was decomposed with aqueous NaOH, acidified, and steam-distilled, the distillate was found to contain only 0.6% of acidic product whereas, when the same procedure was carried out in alcoholic NaOH, the distillate was found to contain 18% of acidic product. However, it has been observed by previous workers (7) that one of the methods of making alpha hydroxyisobutyric acid is by the action of alcoholic alkali on chlorobutanol.

The principal products of degradation of chlorobutanol in aqueous solution, consisting of chloride ion, acetone, and carbon monoxide have been determined quantitatively by several investigators. On the basis of the stoichiometric relationship that each mole of chlorobutanol liberates three Cl^-, chlorobutanol has been quantitatively determined in this study. Sinton (8) and Jensen and Janke (9) have assayed chlorobutanol by determining acetone which is liberated on a mole to mole basis. Bressanin and Segre (10) have quantitatively determined the carbon monoxide liberated during decomposition of chlorobutanol.

The Mechanism of Degradation.—From the data obtained in the study of the degradation of chlorobutanol and the kinetics of its hydrolysis, some aspects of the mechanism of its degradation may be postulated.

[1] Saf-co-meter (carbon monoxide indicator). Developed by National Bureau of Standards, U. S. pat. 2,487,077.

TABLE II.—HALF-LIFE PERIODS, $t^1/_2$, FOR THE DEGRADATION OF CHLOROBUTANOL AT DIFFERENT pH AND AT 25°

pH	$t^1/_2$ (Years)	log $t^1/_2$
2.0	90[a]	1.9542
3.0	90	1.9542
4.0	90[a]	1.9542
5.0	40.3	1.6053
5.5	23.2	1.3655
6.0	9.0	0.9542
6.5	2.4	0.3802
7.0	0.61	−0.2147
7.5	0.23	−0.6383

[a] Calculated assuming that *Ea*, energy of activation, for the degradation of chlorobutanol at pH 2 and 4 is the same as that at pH 3.

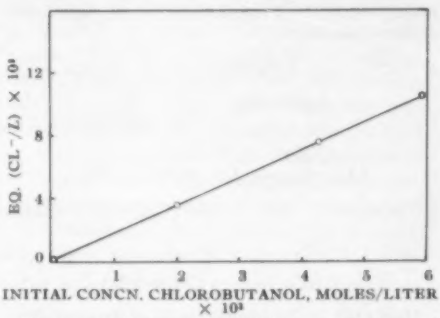


Fig. 8.—Influence of initial concentration of chlorobutanol on amount of chloride ion liberated at pH 6.5 and at 75°. The plot shows the first order dependence of Cl^- formation with respect to chlorobutanol.

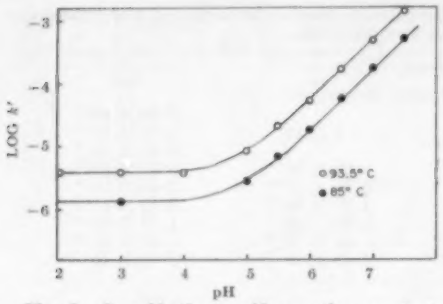


Fig. 9.—Log k', the specific reaction constant, against pH at 93.5 and 85°, showing the order of the reaction with respect to OH^- concentration.

Ray and Basu (1) have suggested the following equations for the hydrolysis of chlorobutanol:

$$(CH_3)_2—C(OH)—CCl_3 \rightarrow (CH_3)_2—C(OH)—COOH + 3\,HCl$$

$$(CH_3)_2—C(OH)—COOH \rightarrow (CH_3)_2CO + CO_2 + H_2O$$

This mechanism does not appear to be correct since carbon dioxide was not identified as one of the decomposition products and since the above equations do not show the liberation of carbon monoxide which is apparently formed during the reaction.

Bressanin and Segre (10) have suggested the following two types of mechanisms for the degradation of chlorobutanol:

1. $(CH_3)_2—C(OH)—CCl_3 \rightarrow (CH_3)_2—C(O)—C—Cl_2 \rightarrow (CH_3)_2CO + CCl_2$

$$CCl_2 \xrightarrow{2OH^-} CO + 2Cl^- + H_2O$$

2. $(CH_3)_2—C(OH)—CCl_3 \longrightarrow (CH_3)_2CO + CHCl_3$

$$CHCl_3 \longrightarrow CO + 3Cl^-$$

Hine (11), in his kinetic study of decomposition of $CHCl_3$ in 66²/₃% aqueous dioxane has proposed the following as the most likely mechanism for its decomposition:

$$CHCl_3 \underset{}{\overset{\text{fast } OH^-}{\rightleftharpoons}} CCl_3^- + H_2O$$

$$CCl_3^- \xrightarrow[\text{slow}]{} CCl_2 + Cl^-$$

$$CCl_2 \xrightarrow[\text{fast}]{OH^- \; HOH} CO + HCO_2^-$$

However, chloroform is only very slowly decomposed in aqueous solution, and in order to effect complete decomposition of $CHCl_3$ it must be refluxed with NaOH for several hours (12). Therefore, it is evident from the kinetic study of the degradation of chlorobutanol liberating acetone, carbon monoxide, and chloride ion that, because the rate of degradation of chloro form inaqueous alkali

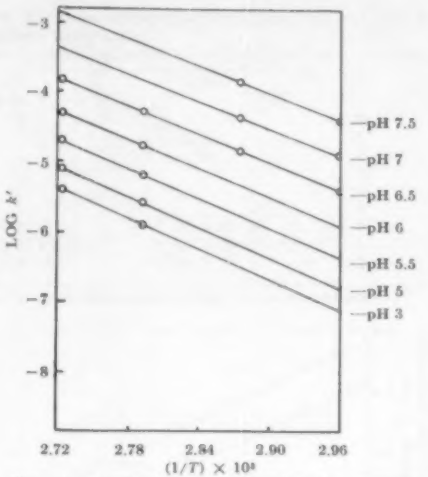


Fig. 10.—Arrhenius plots showing the temperature dependence of rate of degradation of chlorobutanol at different pH.

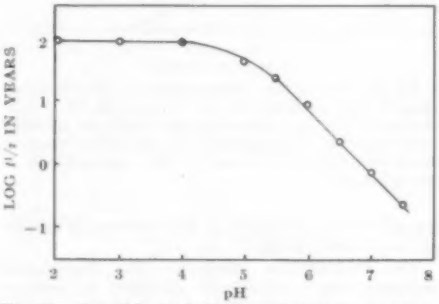


Fig. 11.– Log $t^1/_2$, the half-life period of the reaction, against pH at 25°.

is extremely slow when campared to that of chlorobutanol, chloroform is not an intermediate product during the hydrolysis of chlorobutanol.

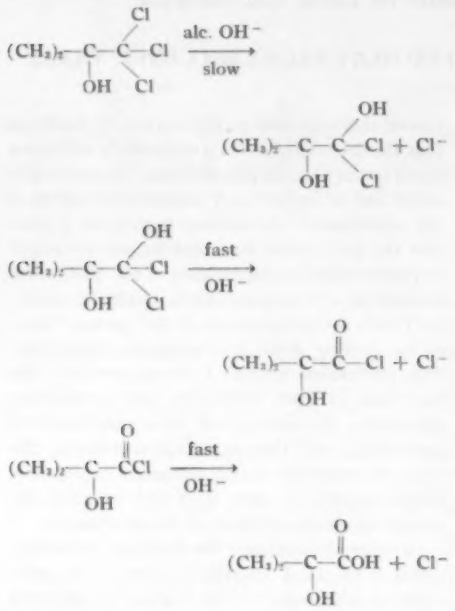
As has been pointed out, the degradation of chlorobutanol is independent of hydrogen ion concentration at low pH but dependent at pH of 5 and above. It is unlikely that two separate mechanisms are involved in this decomposition. The mechanism suggested by Bressanin and Segre (10) appears to be in accord with the available experimental data and is listed below.

$$(CH_3)_2—C(OH)—CCl_3 \xrightarrow[\text{proton transfer}]{OH^- \text{ slow}} (CH_3)_2—C(O)—C—Cl_2 + Cl^-$$

$$(CH_3)_2—C(O)—C—Cl_2 \xrightarrow{\text{fast}} (CH_3)_2CO + CCl_2$$

$$CCl_2 \xrightarrow[\text{fast}]{OH^- \; HOH} CO + 2Cl^- + H_2O$$

Since α-hydroxyisobutyric acid was isolated in minute amounts from the degradation products, its formation is probably due to a competitive reaction as illustrated below:



This type of reaction seems to be highly favored in alcoholic medium (7).

SUMMARY AND CONCLUSIONS

1. The degradation of chlorobutanol in aqueous solutions appears to be a specific hydroxyl-ion-catalyzed reaction. It is first order with respect to chlorobutanol. With respect to OH^-, it is first order from pH 5 to 7.5 and zero order from pH 2 to 4. Although this study was conducted only up to pH 7.5, it would follow that the first order dependency on chlorobutanol would hold true for pH above 7.5.

2. The apparent energy of activation, E_a, for the degradation of chlorobutanol in aqueous solutions from pH 2 to 7.5 was found to be a constant at 30.7 ± 0.8 kilocalories. Since this apparent energy of activation includes the heat of ionization of water (approximately 12 kilocalories), the energy of activation for the hydroxyl reaction would be 18.7 kilocalories, a reasonable value for hydrolytic reactions of this type.

3. The half-lives at 25° for chlorobutanol in aqueous solutions were determined from the calculated specific reaction constants for reactions at various pH and at 25°. The half-life of chlorobutanol in a buffered solution at pH 3 was calculated to be 90 years at 25° whereas its half-life under similar conditions at pH 7.5 was found to be 0.23 years. Similarly, the decomposition of chlorobutanol in aqueous solution at 115° during a thirty minute heating period at pH 5 and at pH 6 were calculated to be 13 and 58 per cent, respectively.

4. It is to be noted that the loss of chlorobutanol from pharmaceuticals is due not only to chemical decomposition but also to the volatilization during preparation and during storage through porous containers, closures, etc.

5. The principal degradation products of chlorobutanol in aqueous solutions were found to be acetone, carbon monoxide, H^+, Cl^-, and a trace amount of α-hydroxyisobutyric acid.

6. Due to the production of H^+ during hydrolysis, the pH of the medium, if not buffered, is apt to be lowered. Because of this lowering of pH and because the rate of degradation is very slow at low pH, the hydrolysis would be somewhat self-limiting in unbuffered solutions.

7. While formulating chlorobutanol in pharmaceuticals, it is desirable to keep the pH of the medium at or below pH 4, since the rate of degradation of chlorobutanol is small and constant at pH 2, 3, and 4. If the pH of the medium is to be kept above pH 4, the amount of chlorobutanol that will be decomposed at that pH during preparation or storage at a particular temperature can be calculated from the data given in this paper and this calculated amount may be added in excess initially, wherever applicable, to compensate for the loss due to degradation.

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Studies on Local Anesthetics XVIII

Some New Quaternary Salts of Basic Carbamates

By ALEŠ SEKERA†, LIBOR NOVÁČEK, JAROSLAV SALAČ, and ČENĚK VRBA‡

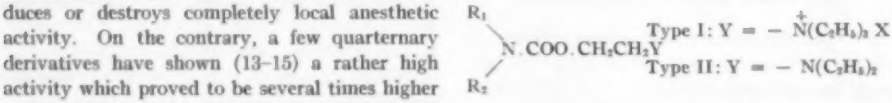
Seven ethiodides and two ethochlorides of diethylaminoethyl esters of substituted carbamic acid were prepared and tested pharmacologically. The activity in both surface and infiltration anesthesia and acute toxicity tests was determined.

IN MOST local anesthetics, e.g., substituted basic benzoates, anilides, acetophenones, arylethers, etc., the hydrophilic part of the molecule is represented by nitrogen whose tertiary form is to be preferred from the point of view of toxicity and local tolerance (substances with primary and secondary amine function are often less advantageous). Compounds including quaternary nitrogen have been neglected lately, on account, very likely, of the generally accepted rule that quaternization destroys anesthetic properties (1, 2).

It appears from studies on local anesthetic effects of compounds containing a quaternary ammonium group that quaternization of cocaine (3–5), procaine (5–8), Xylocaine (7, 9), Stovaine (7), Pontocaine (7), and a whole series of other anesthetics (6, 7, 10–12) in most cases either reduces or destroys completely local anesthetic activity. On the contrary, a few quaternary derivatives have shown (13–15) a rather high activity which proved to be several times higher than that of the corresponding tertiary bases (16, 17). There is thus a disagreement or even frequently the inconsistency between the results of the studies quoted. This might probably be explained by the variety of the pharmacological methods used, and above all, by the type of anesthesia used in tests, because the quaternary salts are generally more effective in both infiltration and conduction anesthesia than in surface anesthesia. The inconsistency between the results may also be explained by a retarded onset of anesthesia, so characteristic of these compounds, which might have prevented some workers from noticing their effect.

The mechanism of local anesthetic action of quaternary ammonium compounds is not yet known although some recent works (18) point out that the decomposition of a quaternary substance into a tertiary base is plausible and this conversion might aid in explaining the anesthetic action of the quaternaries, the tertiary base might constitute the very active form and its activity might be potentialized in the presence of the quaternary derivative. These phenomena might be associated with a depolarization of the nervous fibers or by slowing down of enzymatic metabolism. This mechanism appears to be supported by the fact that in both infiltration and conduction anesthesia, the activity of these substances is generally greater than in surface anesthesia; the type of anesthetic action (retarded onset, prolonged anesthesia with slow and irregular decrease) speak also in favor of this mechanism.

In order to determine the influence of quaternization on local anesthetic activity of basic esters of substituted carbamic acids, we prepared seven ethiodides (S 70–S 76) and two ethochlorides (S 84, S 85) of type I. These are quaternary compounds of seven basic carbamates (II)



chosen as characteristic representatives of the various structural types of compounds described in our previous papers (19–22). The structure of the prepared compounds are shown in Table I. The derivatives S 70 to S 72, whose structures already suggest their anesthetic inactivity, have been included in the studied series because of other pharmacological studies to be carried out.

The iodides S 70 to S 76 were prepared by heating the corresponding diethylaminoethyl esters (19–22) with the excess of ethyl chloride (method A); the chlorides S 85 and S 86 were prepared by a similar process by heating the tertiary basic esters with ethyl chloride in a sealed tube (method B) or from quaternary iodide by action of silver chloride (method C).

In order to carry out an improved investigation of the influence of quaternization on local anesthetic effect, we carried out in each case of active quaternary compound a simultaneous determination of the activity of the tertiary basic esters (S

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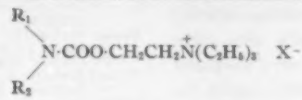
TABLE I.—QUATERNARY SALTS



No.	R_1	R_2	X^-	Method	Yield, %	M. P., °C.	N Calcd., %	N Found, %	X Calcd., %	X Found, %
S 70	H	H	I	A	79	240[a]	8.86	8.64	40.14	40.36
S 71	C_2H_5	H	I	A	66	90	8.14	8.07	36.84	37.14
S 72	C_2H_5	C_2H_5	I	A	65	101	7.52	7.56	34.09	33.75
S 73	C_6H_5	H	I	A	73	131[b]	7.16	7.25	32.43	31.39
S 74	β-$C_{10}H_7$	H	I	A	75	195	6.33	6.49	28.69	28.57
S 75	p-C_4H_9O-C_6H_4	H	I	A	86	97	6.03	5.98	27.33	27.25
S 76	C_6H_5	C_6H_5	I	A	62	228	5.98	6.20	27.09	26.70
S 84	β-$C_{10}H_7$	H	Cl	B	69	202	7.98	7.89	10.10	10.34
S 85	p-C_4H_9O-C_6H_4	H	Cl	B	53	180	7.51	7.59	9.51	9.60
				C	75					

[a] Literature (28, 29) m. p. 205° and 245–246°, resp. [b] Literature (28) m. p. 128°.

TABLE II.—COMPARISON OF LOCAL ANESTHETIC EFFECTS AND OF TOXICITY OF QUATERNARY COMPOUNDS (I) WITH THE CORRESPONDING TERTIARY BASIC ESTERS (II)

Quaternary Compound (I)				Corresponding Tertiary Basic Ester (II)			
	Relative Activity				Relative Activity		
No.	Surface Anesthesia[a]	Infiltration Anesthesia[b]	LD_{50}, mg./Kg.	No.	Surface Anesthesia[a]	Infiltration Anesthesia[b]	LD_{50} mg./Kg.
S 73	Inactive[c]	(0.5–1)[d]	430	S 11	0.1	1.2	300
S 74	Inactive[e]	Inactive[e]	76	S 13	Irritation	1.3	610
S 75	Inactive[f]	Incomplete anesthesia[f]	150	S 46	1.4	3	430
S 76	Incomplete anesthesia	(1–2)[g]	(300)[h]	S 25	1.4	3.6	175
S 84	Irritation[i]	(1–2)[j]	110	...	...	...	...
S 85	0.7	1.9	110	...	...	...	...

[a] Cocaine = 1 (LD_{50} = 125 mg./Kg.). [b] Procaine = 1 (LD_{50} = 630 mg./Kg.). [c] Solution inactive at 2%; no effect on the tongue. [d] Irregular decrease of anesthesia with concentrations $M/5$ to $M/20$. [e] Experiment with saturated solution $M/100$. [f] Experiment with saturated solution $M/30$. [g] Irregular decrease of anesthesia with concentration $M/100$ (slight irritation). [h] Approximative determination (slight solubility: 0.47%). [i] Concentrations between 1 and 5% cause irritation; a 1% solution is inactive. [j] Onset of anesthesia is retarded.

11, S 13, S 46, and S 25), already mentioned (19, 22) and studied by another pharmacological method (23).

EXPERIMENTAL[1]

Diethylaminoethyl Esters of Substituted Carbamic Acids.—These have been prepared by methods described in our previous communications (19–22) and isolated as bases. Esters derived from carbamic, ethyl carbamic, and diethylcarbamic acids have been purified by distillation under reduced pressure. The ester of 4-butoxycarbanilic acid has been recrystallized from petroleum ether. The other bases have been utilized as such.

Quaternary Salts.—*Method A.*—Two-hundredths of a mole of tertiary basic ester are refluxed for one and one-half hours with 15.6 Gm. (0.1 mole) of ethyl iodide. The excess of this was distilled under reduced pressure, the residue was dissolved in absolute alcohol and precipitated by anhydrous ether. The crystals formed were filtered off and crystallized in anhydrous alcohol-ether. In some cases the quaternary compounds precipitated as an oily mass which solidified on standing a few days.

[1] Melting points are not corrected. The analyses have been made in the analytical laboratories (director Dr. Ing. Z. Večeřa) of the Institute of Organic Syntheses, Pardubice-Rybitví, Czechoslovakia.

Method B.—One-hundredth of a mole of diethylaminoethyl ester of the substituted carbamic acid was heated for ten hours in a sealed tube at 80 to 100° with 26 Gm. (28 cc.; 0.4 mole) ethyl chloride. The product of the reaction was treated in the same way as in method A.

Method C.—A solution of 4.6 Gm. (0.01 mole) of substance S 75 (base) in 150 cc. water was shaken for five hours with 2.9 Gm. (0.02 mole) of freshly precipitated silver chloride. After filtration the solution was evaporated under reduced pressure and the residue treated as described in method A.

The yields, constants, and analyses are shown in Table I.

PHARMACOLOGY

The relative activity in surface anesthesia (rabbit cornea, $M/100$ cocaine as standard) and infiltration anesthesia (intradermal application to guinea pigs, $M/50$ procaine as standard) was calculated from the molar concentration, experimentally found to give the same effect as the standard. The method has been described in detail elsewhere (24, 25).

The toxicity was studied according to Kärber (26) by determining the LD_{50} with white mice (strain H) in subcutaneous injection.

The results are presented in Table II.

DISCUSSION AND SUMMARY

By action of alkyl halides on diethylaminoethyl esters of substituted carbamic acids, nine corresponding quaternary salts have been prepared. In this series the effect of quaternization on the activity in surface and infiltration anesthesia have been studied.

The following correlation between molecular structure and pharmacodynamic action can be made:

1. The nonsubstituted derivative S 70 and the aliphatic derivatives S 71 and S 72 are inactive as well as the corresponding tertiary basic esters S 10 (20), S 2 (21), and S 22 (20).

2. Aromatic derivatives (Table II) have been found active, although they showed very slight effects both in surface and infiltration anesthesia. This fact is very probably increased or perhaps even caused by their low water solubility; the anesthetic activity of the chlorides S 85 and S 86, which are more soluble, is also higher.

3. It appears from the comparison between the tertiary and quaternary esters that in this series quaternization lessens the activity in both surface and infiltration anesthesia.

As far as the other pharmacodynamic effects are concerned, it has been found that these substances also have cholinergic (S 70–S 72), spasmolytic (S 75, S 76, S 85, and S 86), and high curare-like activity (the effect of compounds S 73 to S 76, S 84, and S 85 are of the same order as the Flaxedil activity). The detailed results of these experiments will be published at a later date (27).

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Studies on Local Anesthetics XIX*

Substituted Phenylcarbamates of Piperidinopropanediol

By RUDOLF DOFEK, ALEŠ SEKERA†, and ČENĚK VRBA‡

The synthesis of twelve alkoxy- and two alkyl-substituted phenylcarbamates of piperidinopropanediol is reported. All the compounds are effective in both surface and infiltration anesthesia and are relatively nontoxic.

THE LOCAL ANESTHETIC effects of a number of arylcarbamates derived from basic propanediols has been studied in particular by Rider and his co-workers (1–4). Of this series, Diothane, 3-(1-piperidyl)-1,2-propanediol dicarbanilate hydrochloride has found practical application; the compound is also recorded in "New and Nonofficial Remedies." In the derivatives hitherto recorded, modifications have been carried out mainly in the basic component of the molecule, and the substitution of the aromatic nucleus of the carbanilate moiety has received relatively little attention.

Working with series of diethylaminoethyl esters derived from substituted carbanilic (5–8) and diphenylcarbamic acids (9), we have found that methylation and particularly alkoxylation exerts a favorable effect on the pharmacodynamic ac-

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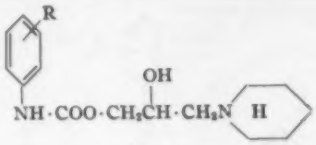
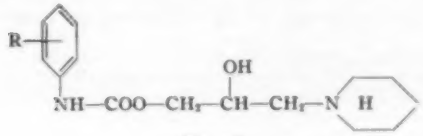
TABLE I.—SUBSTITUTED PIPERIDINOPROPANEDIOL MONOPHENYLCARBAMATE HYDROCHLORIDES



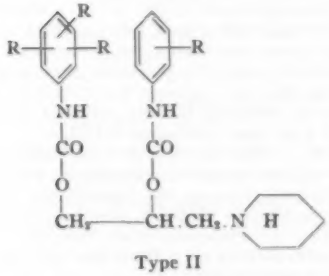
No.	R	M. p., °C.	Solubility, H_2O, %	Yield, %	N Calcd., %	N Found, %	Cl Calcd., %	Cl Found, %
S 100	H	176[a]	50	91	8.90	8.99	11.26	11.11
(Monothane) picrate		172			13.80	13.70		
S 111	2-CH_3O	167–169	50	89	8.18	8.29	10.35	10.38
picrate		164–165			13.08	13.06		
S 112	2-C_2H_5O	144–145	(35–45)	90	7.80	7.77	9.88	9.82
picrate		138			12.70	12.72		
S 102	2-C_4H_9O	102	34	78	7.24	7.14	9.16	9.15
picrate		92–94			12.08	12.08		
S 103	3-C_4H_9O	137	(35–45)	93	7.24	7.14	9.16	9.04
picrate		126			12.08	12.09		
S 104[b]	4-C_4H_9O	175	35	88	7.24	7.26	9.16	9.07
picrate		108			12.08	11.88		

[a] Literature (1) m. p. 176–177°. [b] The crystalline base melts at 106.5° (ethanol). *Anal.*—Calcd.for $C_{19}H_{24}O_4N_2$: C, 65.11 H, 8.63; N, 7.99. Found: C, 65.00; H, 8.62; N, 7.95.

tivity of compounds of this type. In an effort to extend these findings to esters of piperidinopropanediol we have now prepared a series of mono and diesters derived from this diol and alkyl- and alkoxy-substituted phenylcarbamic acids (Types I and II).

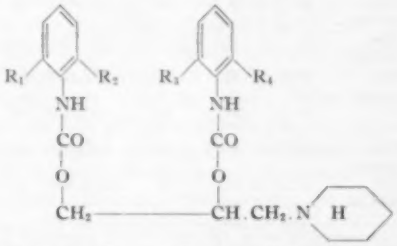


Type I



Type II

For the preparation of these compounds, the usual reaction of piperidinopropanediol with aromatic isocyanates was used. The use of solvent anhydrous ether as described by Rider did not prove suitable in our case because of the low reactivity of the substituted phenyl isocyanates; however, good yields of the products were obtained by refluxing the components in anhydrous benzene. Attempts to prepare diesters of types III to VI, derived from *o*-methyl or *o*-alkoxy-sub-



Type	R_1	R_2	R_3	R_4
III	CH_3	CH_3	CH_3	CH_3
IV	OR	H	H	H
V	H	H	OR	H
VI	OR	H	OR	H

stituted isocyanates, were unsuccessful, presumably because of steric hindrance effects; only monosubstituted derivatives of the type I were isolated in each case.

EXPERIMENTAL[1]

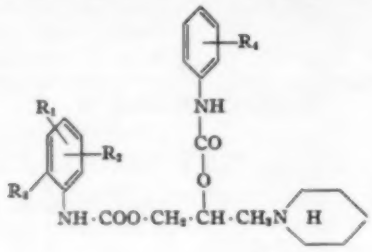
3-(1-Piperidyl)-propanediol-1,2—This was prepared from 3-chloropropanediol-1,2 (10) and piperidine (11) and also from 2,3-epoxypropanol as an intermediate (12). Both methods under suitable conditions gave 50–60% yields but the second proved more reproducible, providing the temperature during the isolation of the epoxypropanol was kept as low as possible (b. p. *ca.* 30–60°).

Aromatic Isocyanates.—These were obtained by the method previously described (5–7), involving the reaction of the appropriate aromatic amines with excess of phosgene in boiling toluene. The yields and physical constants were as previously given (5–8).

Piperidinopropanediol Monophenylcarbamate Hydrochlorides (I).—To a boiling solution of 15.9

[1] All melting points are corrected and were obtained on the Kofler block. Microanalyses were carried out by Mrs. Kleinová-Parolková.

TABLE II.—SUBSTITUTED PIPERIDINOPROPANEDIOL BIS-PHENYLCARBAMATE HYDROCHLORIDES



No.	R_1	R_2	R_3	R_4	Method	M. p., °C.	Solubility H_2O, %	Yield, %	N Calcd., %	N Found, %	Cl Calcd., %	Cl Found, %
S 101	H	H	H	H	A							
(Diothane)						198[a]	1	92	9.68	9.56	8.17	8.20
picrate						118–120			13.41	13.34		
S 117[b]	2-CH_3	6-CH_3	H	H	B	200	0.8	95	9.09	9.08	7.67	7.63
picrate						202 dec.[c]			12.85	13.10		
S 118	2-CH_3	4-CH_3	6-CH_3	H	B	203	0.6	88	8.82	8.95	7.45	7.40
picrate						206[c]			12.58	12.69		
S 105	3-C_4H_9O	H	H	H	B	191–192	0.17	95	8.30	8.13	7.00	6.96
picrate						81–82			12.03	11.94		
S 114	H	H	H	3-CH_3O	C	109–110	3.6	90	9.05	8.97	7.64	7.56
picrate						168[d]			12.80	12.72		
S 116	H	H	H	3-C_2H_5O	C	125–127	1	95	8.79	8.79	7.41	7.38
picrate						184[d]			12.53	12.30		
S 106	H	H	H	3-C_4H_9O	C	134–136	0.3	91	8.30	8.30	7.00	7.09
picrate						174			12.03	11.89		
S 107	H	H	H	4-C_4H_9O	C	186	0.25	87	8.30	8.31	7.00	7.19
picrate						129			12.03	12.03		
S 109	3-C_4H_9O	H	H	3-C_4H_9O	A	153	0.16	88	7.26	7.38	6.13	6.28
picrate						126			10.90	10.81		
S 110	4-C_4H_9O	H	H	4-C_4H_9O	A	173	0.16	83	7.26	7.11	6.13	6.25
picrate						175			10.90	10.71		

[a] Literature (1) m. p. 197–198°. [b] The crystalline base melts at 137–138° (ether). *Anal.*—Calcd. for $C_{28}H_{32}O_4N_2$: C, 67.60; H, 7.30; N 9.88. Found: C, 67.94; H, 7.43; N, 9.67. [c] From dioxane. [d] From acetone. [e] The crystalline base melts at 143° (ethanol). *Anal.*—Calcd. for $C_{30}H_{36}O_6N_2$: C, 66.51; H, 8.99; N, 7.75. Found: C, 67.21; H, 8.19; N, 7.69.

Gm. (0.1 mole) of piperidinopropanediol in anhydrous benzene, 0.1 mole of the appropriate isocyanate was added as a 20% solution in anhydrous benzene. The mixture was refluxed for thirty minutes, the benzene distilled off, the basic ester taken up in 500 cc. of anhydrous ether and precipitated as the hydrochloride by addition of one equivalent of ethereal hydrogen chloride. The crystalline hydrochlorides were washed with dry ether to neutral reaction (three to four portions of 100 cc. each). The hydrochlorides precipitated as oils were obtained crystalline by being kept *in vacuo* over P_2O_5 and KOH for several days; they were then ground and washed with anhydrous ether. The products were recrystallized from ethanol-ether after treatment with activated carbon.

The picrates were crystallized from 96% ethanol.

The melting points, yields, and analyses of the products are given in Table I.

Piperidinopropanediol Bisphenylcarbamate Hydrochlorides (II).—*Method A.*—Derivatives substituted in both benzene rings were prepared with 0.2 moles of the appropriate aryl isocyanate by the procedure used in the preparation of the monophenylcarbamates.

Method B.—Derivatives carrying a substituent in the phenylcarbamate residue attached to the primary alcoholic group of the piperidinopropanediol were prepared by treating 15.9 Gm. (0.1 mole) of piperidinopropanediol in 250 cc. of boiling anhydrous benzene with a 20% solution of 0.1 mole of the appropriate substituted phenylisocyanate in the same solvent, refluxing for thirty minutes, adding 11.9 Gm. (0.1 mole) of phenylisocyanate as a 20% solution in anhydrous benzene, refluxing for thirty minutes more, and working up as before.

Method C.—Derivatives carrying a substituent in the phenylcarbamate residue attached to the secondary hydroxyl group of the piperidinopropanediol were prepared as in *Method B*, except that the order of addition of the isocyanates was reversed.

The picrates were crystallized from 96% ethanol unless otherwise stated.

The methods of preparation, melting points, yields, and analyses of the products are given in Table II.

The solubilities of the hydrochlorides were determined by the mercurimetric titration of chloride ion in solutions saturated at room temperature. The results are given in Tables I and II.

PHARMACOLOGY[2]

The relative activity of the compounds in surface anesthesia (rabbit cornea, *M*/100 cocaine as stand-

[2] We are obliged to Miss S. Formáčková for technical assistance with the pharmacological tests.

TABLE III.—PHARMACOLOGICAL PROPERTIES

No.	Anesthetic Activity: Surface Anesthesia	Anesthetic Activity: Infiltration Anesthesia	LD_{50} mg./Kg.	Local Tolerance[a]: Probable Clinical Concn.: Conjunctiva, % (anesth.[b])	Local Tolerance[a]: Probable Clinical Concn.: Subcutaneous, %	Irritation Threshold: Conjunctiva, %	Irritation Threshold: Subcutaneous, %
S 100	0.46	3.0	790	4 (±) (60)	2+	4	1.5
S 101	2.9	4.8	890	1 (±) (70)	1+	1	0.5
S 102	8.8	16.5	127	0.2 (±) (60)	0.14±	0.2	0.2
S 103	18	14	105	0.2 − (120)	0.1±	0.4	0.1
S 104	5	6	430	0.2 − (60)	0.6 +	0.4	0.5
S 105	9.4	5–6	Spar.[c] solub.	...	...	...	...
S 106	7.7	7.7	Spar.[d] solub.	...	...	...	...
S 111	0.52	1.4	920	...	...	...	...
S 112	1.2	2.7	690	4 − (45)	2 ++(+)	6	1
S 114	2.2	7.2	1,340	1 (±) (60)	1.4 +	1	0.2
S 116	6.9	16.5	Spar.[e] solub.	...	0.28 (±)	...	0.28
S 117	17	5.5	430	0.05 ± (35)	0.4 +	0.05	0.2
S 118	13	13.5	Spar.[f] solub.	0.5 (±) (60)	0.5	0.5 +	50.
Cocaine	1	3.6	125	5 − (40)	0.1 − 1	10	1–2
Procaine	0.15	1	630	10 − (30)	1 − 4	15	4

[a] − No irritation, + moderate irritation, ++ evident irritation, +++ strong irritation. [b] Duration of complete anesthesia (minutes). [c] $LD_5 \geq 120$ (saturated solution). [d] $LD_5 \geq 220$ (saturated solution). [e] $LD_5 \geq 600$ (saturated solution). [f] $LD_5 \geq 400$ (saturated solution).

ard) and infiltration anesthesia (intradermal application to guinea pigs, *M*/50 procaine as standard) was calculated from the molar concentration experimentally found to give the same effect as the standard. The method has been described in detail by Vrba and Sekera (13) and Roth (14).

The toxicity was studied according to Kärber (15) by determining the LD_{50} in white mice (strain H) by subcutaneous application. The low solubility of some of the compounds did not permit the determination.

Local tolerance was studied in rabbits by application into the conjunctival sac and by subcutaneous injection at the root of the ear, and determining the threshold concentration causing local irritation. The tolerance of clinically probable doses was also studied. The results are given in Table III.

DISCUSSION AND SUMMARY

Sixteen derivatives of the Diothane series were prepared and tested for local anesthetic activities, toxicity, and irritation.

The following correlation between molecular structure and anesthetic activity can be made:

1. Ethoxylation and butoxylation of the benzene nucleus of carbanilic acid increases the activity of the esters of piperidinopropanediol. The same fact has already been observed previously in the case of simple basic esters (5–7, 9).

In the series of Diothane derivatives, less soluble in water than the derivatives of Monothane, the lipophilizaion of the molecule by monobutoxylation seems to be the limit; this appears for example from the results of tests of the slightly soluble substances S 107, S 109, and S 110, whose aqueous saturated solutions proved to be inactive.

2. Methoxylation is the least advantageous. In most cases the activity increases in proportion with the number of carbons of alkoxyl.

When the substitution of the benzene nucleus is considered, the *ortho* and *meta* positions are more advantageous than the *para*. These results agree as well with those obtained in our previous studies relating to simple basic carbamates (5–7).

3. Methylation of the benzene nucleus in the Diothane molecule highly increases the activity of surface and infiltration anesthesia.

In accordance with the pharmacological experiments which have been undertaken, several of the derivatives studied have proved to be more advantageous than the pattern substance, Diothane, especially from the point of view of surface anesthesia. Some of the more promising compounds are being tested clinically.

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Studies on Local Anesthetics XXI*

Some Derivatives of ω-Diethylaminoacetanilide

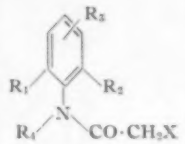
By ALOIS BOROVANSKÝ, ALEŠ SEKERA,† and ČENĚK VRBA‡

The preparation, surface, and infiltration activities and the toxicity of diethylaminoacetanilide and seven of its derivatives are described.

IN OUR EARLIER PUBLICATIONS we studied chiefly the basic esters of substituted carbamic acids. By appropriate structural modifications we succeeded in preparing several very active, often slightly toxic compounds (1, 2). Several of these, in spite of their very favorable therapeutic index, proved themselves unsuitable for clinical trials because of their poor local tolerance (irritation).

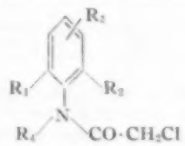
From the supposition that this undesirable property results chiefly from the formation of aromatic amine by the metabolism of the active carbanilate, we began studies of the substances, structures of which allowed us to suppose that the formation of aromatic amine would be decreased and, eventually, completely excluded. In the choice of new structural types, in order to be able even in these new series to apply our knowledge of relations between the structures, the physicochemical properties, and the anesthetic activity of the basic carbanilates, we have knowingly directed our attention to the compounds having structures similar to the substances previously studied. Our first work in that area was the preparation of basic alkoxy-propiophenones of the Falicaine series (3, 4) and of basic carbanilates (5, 6) of which the stability was augmented by (group —NH·COO—) the methylation of the benzene nucleus.

Continuing these studies, we decided to direct our attention to a closely related series—the basic anilides, on the supposition that the group Ar·NH·CO·R would be more slowly metabolized in the alkaline medium of human tissue than the Ar·NH·COO·R group. For the fundamental orientation in this new series, we prepared eight derivatives of type II; the description of the preparation and the results of the pharmacological experiments make the subject of this communication. Some substances have already been described in the literature (7–9) but nevertheless we have included them in our series in order to be able to study all together, utilizing the same pharmacological technique.



Type I, X = Cl
Type II, X = $N(C_2H_5)_2$
R_1, R_3 = H, CH_3, C_4H_9O;
R_2 = H, CH_3; R_4 = H, C_6H_5

TABLE I.—SUBSTITUTED CHLOROACETANILIDES



No.	R_1	R_2	R_3	R_4	Yield, %	M. p., °C.
1	H	H	H	H	79	135[a]
2	CH_3	H	H	H	78	108–109[b]
3	CH_3	CH_3	H	H	79	142[c]
4	CH_3	CH_3	4-CH_3	H	74	172[d]
5	H	H	H	C_6H_5	90	119–121[e]
6	C_4H_9O	H	H	H	96	54[f]
7	H	H	3-C_4H_9O	H	82	94–95[g]
8	H	H	4-C_4H_9O	H	96	123–125[h]

[a] Dimroth (13) m. p. 134–135°. [b] Literature (14) m. p. 111–112°. [c] Literature (15) m. p. 145–146°. [d] Literature (15) m. p. 178–179°. [e] Literature (16) m. p. 118°. [f] *Anal.*—Calcd. for $C_{13}H_{18}NO_2Cl$ (241.7): N, 5.80. Found: N, 5.94. [g] *Anal.*—Calcd. for $C_{21}H_{28}NO_2Cl$ (241.7): N, 5.80. Found: N, 5.82. [h] Literature (9) m. p. 132.5°.

The structure of the substances studied can be seen in Table II. For their preparation we have utilized the method (8) starting from the corresponding aromatic amines which, passing through the chloroacetanilides (I), have supplied, in the reaction with diethylamine, the final basic anilides.

EXPERIMENTAL[1]

Mesidine.—This was prepared (5) by the catalytic hydrogenation of nitromesitylene over Raney nickel (initial pressure 30 Atm.).

Butoxyanilines.—A similar mode of reduction was

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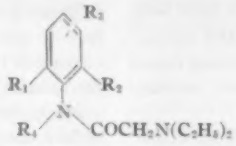
[1] All melting points were determined on a Kofler block and are corrected. Microanalyses were carried out by Mrs. Kleinová-Parolková.

TABLE II.—SUBSTITUTED DIETHYLAMINOACETANILIDES



No.	R_1	R_2	R_3	R_4	Base: Yield, %	Base: b. p., °C./mm.	Base: m. p., °C.	Hydrochloride: m. p., °C.	Hydrochloride: N Calcd., %	Hydrochloride: N Found, %	Hydrochloride: Cl Calcd., %	Hydrochloride: Cl Found, %
S 200	H	H	H	H	72	140–141/1.5[a]	...	108–109	11.54	11.38	14.61	14.39
S 201	CH_3	H	H	H	59	141.5/0.6[b]	...	120–122[c]	10.91	10.99	13.81	13.77
S 202	CH_3	CH_3	H	H	61	159/1[d]	70[d]	116–118[d]	9.70	9.62	12.28	12.24
S 203	CH_3	CH_3	4-CH_3	H	66	154–155/0.6[f]	...	140[g]	9.84	9.94	12.45	12.47
S 210	H	H	H	C_6H_5	88	175/1[h]	...	145–146[i]	8.79	8.49	11.12	11.36
S 220	C_4H_9O	H	H	H	56	182–183/0.7	...	118–120	8.90	8.80	11.26	11.14
S 221	H	H	3-C_4H_9O	H	45	186–187/0.5	...	123–125	8.90	9.10	11.26	11.14
S 222	H	H	4-C_4H_9O	H	53	185/0.5[j]	43–45	152–154[k]	8.90	8.78	11.26	11.10

[a] Literature (7) b. p. 116–117°/0.15 mm. [b] Literature (7) b. p. 159–160°/0.1 mm. [c] From acetone. [d] Literature (8) b. p. 159–160°/2 mm., m. p. 67°. [e] Sample for analysis dried over P_2O_5 by 110°/0.2 mm. *Anal.*—Calcd. for $C_{14}H_{22}ON_2Cl \cdot H_2O$ (288.8): C, 58.22; H, 8.02. Found: C, 58.51; H, 8.39. Even after prolonged drying under these conditions we were not able to prepare the anhydrous hydrochloride, described by Löfgren. [f] Literature (8) b. p. 155–156°/0.6 mm. [g] From acetone. Sample for analysis dried over P_2O_5 by 110°/0.2 mm. Literature (8) m. p. 136–137°. [h] Literature (8) b. p. 194°/3 mm. [i] *Anal.*—Calcd. for $C_{18}H_{23}ON_2Cl$ (318.9): C, 67.80; H, 7.27. Found: C, 67.70; H, 7.31. [j] Literature (9) b. p. 186°/0.5 mm. [k] Literature (9) m. p. 153°.

used in the preparation of these substances from the aromatic nitro-derivatives which were synthetized using the method described in one of our previous communications (1).

Chloroacetanilides (I).—In a solution of 0.3 mole of aromatic amine in 180 cc. anhydrous acetone 60 Gm. (excess *ca.* 80%) of the acid chloride of monochloroacetic acid was introduced in small portions. The reaction completed, the mixture was, after one hour of settling, poured into 300 cc. of hydrochloric acid (8%). The crystals of the chloroacetanilide were separated after twenty-four hours and washed free of acid.

The N-chloroacetyldiphenylamine was prepared from the diphenylamine and the chloride of the monochloroacetic acid in boiling toluene (8).

The samples for analysis were recrystallized from diluted alcohol. The yields and constants of the prepared products are reported in Table I.

Diethylaminoacetanilides (II; S 200–S 222).—0.2 Mole of chloroacetanilides was refluxed for six hours with 37 Gm. (0.5 mole) of diethylamine in 100 cc. of anhydrous benzene. The hydrochloride of diethylamine formed in the reaction was filtered off and the benzene solution washed twice with two 50-cc. portions of water. The solvent was removed by distillation. The residue was dissolved in 100 cc. of hydrochloric acid (10%), and the solution extracted twice by 70 cc. of ether. The acid solution was made alkaline by treatment with ammonia solution in order to liberate the base. This was extracted by ether (four times, 50 cc.) and the ethereal solution, dried on sodium carbonate, was fractionated.

The substance S 210 was prepared in anhydrous ether by heating for four hours (8).

The hydrochlorides were prepared in anhydrous ether. In the case of substance S 202 the isolation of a better defined crystalline base proved advantageous.

The yields, constants, and analyses of the prepared substances are collected in Table II.

PHARMACOLOGY[2]

The relative activity of the compounds in surface anesthesia (rabbit cornea, *M*/100 cocaine as standard) and infiltration anesthesia (intradermal application to guinea pigs, *M*/50 procaine as standard) was calculated from the molar concentration, experimentally found to give the same effect as the standard. The method has been described in detail by Vrba and Sekera (10) and Roth (11).

The toxicity was studied according to the method of Kärber (12) by determining the LD_{50} in white mice (strain H) by subcutaneous injection. The units are presented in Table III.

DISCUSSION AND SUMMARY

The preparation and the results of the tests of surface and infiltration anesthesia and the toxic-

TABLE III.—PHARMACOLOGY OF SUBSTITUTED DIETHYLAMINOACETANILIDES

Substance	Relative Activity: Surface Anesthesia	Relative Activity: Infiltration Anesthesia	LD_{50} s. c. mg./Kg.
S 200	Incomplete anesthesia[a]	0.12	890
S 201	<0.06[b]	0.24	450
S 202 (Xylocaine)	0.24	1.4	365
S 203 (Mesokain)	1	2.5	295
S 210	0.03	7.4	290
S 220	0.58	0.2	1,930
S 221	7.5	9	170
S 222	3	5.5	550
Cocaine	1	3.6	125
Procaine	0.15	1	630

[a] After application of *M*/3 solution. [b] After application of *M*/3 solution complete anesthesia of 4.5 ± 2 minutes.

[2] We are obliged to Miss M. Perníková and I. Růžicková for technical assistance with the pharmacological tests.

ity of eight basic anilides of the Xylocaine series have been described. The compounds have been shown to be effective in both surface and infiltration anesthesia; some of these have been found to be more active than the standards, namely, cocaine and procaine.

The following correlation between molecular structure and pharmacodynamic action (Table III) can be made:

1. The methylation of the benzene nucleus of diethylaminoacetanilide in the *ortho* position increases the surface and infiltration anesthetic activities. This augmentation of anesthetic effect can perhaps be explained, in addition to the influence of the physicochemical properties, by the increased stability of the anilidic group (Ar·NH·CO·R), which we have already mentioned in a previous communication (6).

2. As a result of the comparison of the effectuated tests of the activity and toxicity, it would seem that Mesokain (S 203) is more advantageous than Xylocaine (S 202).

3. Interesting also is the derivative S 210 which is seven times more active than procaine in surface anesthesia and is only twice more toxic. Surprising is its slight activity in surface anesthesia.

4. Butoxylation of the benzene nucleus of diethylaminoacetanilide increased surface and infiltration anesthetic activity in the series *ortho* < *para* < *meta*. Parallel to this can be noticed an increase in the toxicity. The most advantageous therapeutic index is that of substance S 222 which is three times more active than cocaine in surface anesthesia, five and one-half times more active than procaine in infiltration anesthesia, and only slightly more toxic than procaine.

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A Comparison of the Hypotensive Effects of the Monophosphoric Acid Ester of Thiamine and Thiamine Hydrochloride*

By NATHAN WATZMAN†, JOHN J. DeFEO‡, and JOSEPH P. BUCKLEY

The hypotensive effects of the monophosphoric acid ester of thiamine (RO 1-4788) and thiamine hydrochloride were investigated in anesthetized rats and dogs. The depressor effect of thiamine hydrochloride was 40.8% greater than that of RO 1-4788 in the rat and 42.8% greater than RO 1-4788 in the dog.

IN RECENT YEARS thiamine hydrochloride has created a new problem for the practicing physician. Clinicians (1–3) have reported that parenterally administered thiamine hydrochloride has produced peripheral circulatory collapse and a shock syndrome similar to that produced by vasodepressors and allergenic agents. Reingold and Webb (4) reported a death following an intravenous administration of 100 mg. of thiamine hydrochloride. Laboratory investigators have corroborated these findings in many species of animals (5–6). Mazzella (7) reported that 50 mg./Kg., i. v., of thiamine hydrochloride produced arterial hypotension and bradycardia in the sympathectomized dog. These effects were still evident even after ganglionic blockade with tetraethylammonium chloride or adrenergic blockade with dibenamine indicating the possibility of a direct vascular depressant action. Smith, *et al.* (5), demonstrated, through perfusion of the mesenteric and femoral arteries of the dog and the isolated ear vein of the rabbit, that the vasodilatory property of thiamine hydrochloride was not necessarily a direct peripheral action but possibly central, involving depression of the vaso-

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constrictor centers. Wolfson and Ellis (8) reported that the vasodepressor effect of thiamine did not appear to be due to a direct action on blood vessels.

French investigators (9) reported that the pyrimidine portion of the molecule was responsible for the visible toxic symptoms. In a later paper (10) they suggested the possibility that excess thiamine produced too great a decarboxylation of pyruvic acid and that this disturbance in metabolism might be responsible for the resulting "thiamine shock." They reported a definite protection against the hypotensive activity of thiamine hydrochloride with 3 Gm./Kg. sodium pyruvate, i. v., in the rabbit.

Since the monophosphoric acid ester of thiamine had been found to be as active as thiamine hydrochloride in vitamin B_1-deficient animals (11) it seemed desirable to compare the hypotensive and vascular effects of the two compounds (Fig. 1).

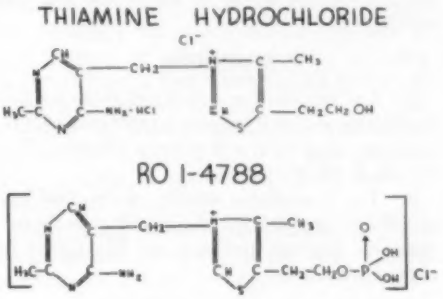

Fig. 1—Structural formulas of the compounds studied.

METHODS

Hypotensive Activity in Rats.—Wistar rats, weighing between 150 and 200 Gm., were anesthetized with 1.2 Gm./Kg. of urethan intraperitoneally and direct carotid blood pressure recordings were obtained as previously described (12). Varying doses of thiamine hydrochloride (5–50 mg./Kg.) were administered intravenously to a total of 26 rats to obtain the minimal dose producing a marked hypotensive response. The selected dose of thiamine hydrochloride (20 mg./Kg.) was administered intravenously to 30 rats (13 males, 17 females) and the equimolar dose (23.6 mg./Kg.) of the monophosphoric acid ester of thiamine, henceforth designated as RO 1-4788, to another series of 30 rats (13 males, 17 females).

Hypotensive Activity in Dogs.—Mongrel dogs were anesthetized with pentobarbital sodium, 35 mg./Kg., administered intravenously. The left carotid blood pressure was recorded with a mercury manometer or a Sanborn Electromanometer. The experiment was designed so that each animal served as its own control. The test consisted of administering thiamine hydrochloride (10.0 mg./Kg.) and RO 1-4788 (11.8 mg./Kg.) alternately at three-hour intervals in a series of three doses to 20 dogs. The sequence of dosage was reversed in 50% of the experiments. Two per cent solutions of thiamine hydrochloride and RO 1-4788, having a pH of 3.3 and 3.1, respectively, were used. In some of the experiments both derivatives were buffered to a pH of 7 with disodium phosphate.

The effects of pretreatment with sodium pyruvate were investigated in three other dogs. A dose of 5, 10, or 20 mg./Kg. of thiamine hydrochloride was administered via the femoral vein. Sodium pyruvate, 1 Gm./Kg., was administered one hour after the test dose of thiamine, and two minutes later the test dose of thiamine hydrochloride was repeated.

Peripheral Vascular Effects.—The effects of the intravenous administration of equimolar doses of the two compounds on the mesenteric vascular bed were studied in the rat meso-appendix preparation (13). The diameter of one of the magnified arterioles in the field was measured in mm. units prior to and immediately after drug administration. A total of 16 rats was utilized for this study.

RESULTS

Hypotensive Activity in Rats.—The effects of equimolar doses of thiamine hydrochloride and RO 1-4788 on the blood pressure of the rat are summarized in Table I. The hypotensive activity of thiamine hydrochloride was 40.8% greater than that produced by the monophosphoric acid ester. The difference between the responses was statistically significant at the 0.001 level when the data were subjected to the "Student" t test. There was an apparent sex variation in response in that the difference between the mean responses in the males was highly significant ($P < 0.001$) whereas the difference in responses in the female rat was relatively insignificant ($P < 0.1$). The mean duration of action of thiamine hydrochloride and RO 1-4788 was one hundred and sixty-five and one hundred and seventy-seven seconds, respectively.

TABLE I.—THE EFFECTS OF THIAMINE HYDROCHLORIDE AND RO 1-4788 ON THE BLOOD PRESSURE OF THE RAT

Sex	No.	Drop in Blood Pressure, Mean % Thiamine HCl	RO 1-4788	t Test	P[a]
Mixed	60	31.1 ± 1.3	22.3 ± 1.6	4.3	<0.001
M	26	32.8 ± 2.03	19.4 ± 2.48	4.2	<0.001
F	34	29.8 ± 1.8	24.5 ± 2.1	1.96	<0.1

Mean ± standard error of the mean.
[a] Significance of difference between groups as determined by Student's t test.

Hypotensive Activity in Dogs.—Thiamine hydrochloride elicited a hypotensive response in dogs which was 42.8% greater than that produced by RO 1-4788 (Figs. 2 and 3, Table II). A variation in sex response was also noted in dogs since the ratio of the mean responses of RO 1-4788 to thiamine hydrochloride was 0.54 in males and 0.78 in females. The buffered solutions of both compounds elicited the characteristic hypotensive effects observed with the unbuffered solutions.

Pretreatment of the dogs with 1 Gm./Kg. of sodium pyruvate partially blocked the hypotensive activity of thiamine hydrochloride. The hypo-

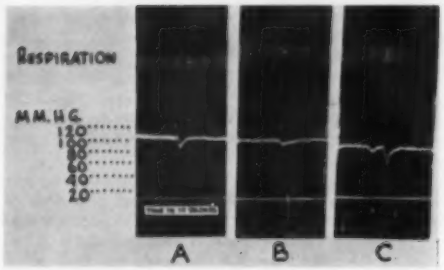

Fig. 2.—The effects of thiamine hydrochloride and RO 1-4788 on the blood pressure of a normotensive dog, male, 5.8 Kg. A and C—effect of 10.0 mg./Kg. of thiamine hydrochloride; B—effect of 11.8 mg./Kg. of RO 1-4788.

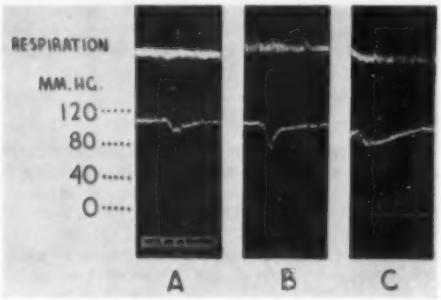

Fig. 3.—The effects of thiamine hydrochloride and RO 1-4788 on the blood pressure of a normotensive dog, male, 9.07 Kg. A and C—effect of 11.8 mg./Kg. of RO 1-4788; B—effect of 10.0 mg./Kg. of thiamine hydrochloride.

tensive activity of 5 mg./Kg. was reduced 25.7%, 10 mg./Kg. reduced 53.0%, and 20 mg./Kg. reduced 22.7%.

Peripheral Vascular Effects.—Equimolar doses of the two thiamine derivatives (10.0 mg./Kg. of thiamine hydrochloride and 11.8 mg./Kg. of RO 1-4788) increased the size of the mesenteric vascular bed slightly in 8 of 16 preparations, however there was no apparent difference in the effects elicited by the two compounds.

DISCUSSION

There was a very close similarity in the hypotensive activity of the compounds in rats and dogs. The hypotensive activity of thiamine hydrochloride was 40.8% greater than RO 1-4788 in the rat and 42.8% greater than RO 1-4788 in the dog when equimolar doses were tested. The variation in sex response was also similar in rats and dogs in that the difference in mean responses in the male was much greater than the difference observed in females. The difference in the mean responses in the male rat was statistically highly significant whereas the difference in means of the female was not significant at the 0.05 level ($P < 0.1, > 0.05$).

TABLE II.—THE COMPARATIVE EFFECTS OF THIAMINE HYDROCHLORIDE AND RO 1-4788 ON THE BLOOD PRESSURE OF NORMOTENSIVE DOGS

Male			Female		
Drop, Mean % Thiamine HCl	Drop, Mean % RO 1-4788	Ratio[a]	Drop, Mean % Thiamine HCl	Drop, Mean % RO 1-4788	Ratio[a]
13.2	6.4	0.48	9.4	10.3	1.10
9.7	4.9	0.51	27.4	5.2	0.19
29.3	7.0	0.24	19.4	9.1	0.47
47.2	25.4	0.54	12.6	12.5	0.99
17.7	20.6	1.16	10.3	8.5	0.83
23.4	7.2	0.31	16.8	11.4	0.68
22.2	12.3	0.55	11.0	11.8	1.07
		$\bar{X}$ 0.54	18.4	8.7	0.47
			13.4	13.0	0.90
			44.2	23.7	0.53
			10.4	17.9	1.72
			8.5	4.1	0.48
			13.7	10.5	0.76
					$\bar{X}$ 0.78

[a] RO 1-4788: thiamine HCl.

SUMMARY

1. The hypotensive activity of thiamine hydrochloride was 40.8 per cent greater than that of the monophosphoric acid ester of thiamine (RO 1-4788) in the Wistar rat.

2. The hypotensive activity of thiamine hydrochloride was 42.8 per cent greater than the hypotensive response produced by RO 1-4788 in normotensive dogs.

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Separation of Decomposed Products of Phenobarbital Sodium by Paper Chromatographic Technique*

By A. J. KAPADIA, J. E. GOYAN, and J. AUTIAN

The decomposed products of phenobarbital sodium have been reported as consisting of phenylethylacetyl urea, phenylethyl acetic acid, and urea. Several solvent systems have been suggested for use in separating the various barbiturates from mixtures by paper chromatographic procedures. A paper chromatographic method has been developed to separate and identify the various decomposed products of phenobarbital sodium in aqueous solutions.

THE DEGRADATION of the barbiturates in alkaline solutions is well known to the pharmaceutical practitioner. One of the earliest references to the instability of malonyl urea derivatives was reported in 1903 (1). It was found that diethylacetyl urea was formed on heating barbital solutions. Husa and Jatul (2) have summarized the pathways of decomposition of the barbiturates in alkaline solutions. Rotondaro (3) has separated and identified the decomposition products of phenobarbital in alkaline solutions as being phenylethylacetyl urea which further hydrolyzes to produce phenylethyl acetic acid and urea. Fretwurst (4) studied the stability of seventeen barbiturates in alkaline solutions and quantitatively determined the decomposition products of the various drugs.

Even though chromatographic techniques have been employed for separating the various barbiturates from each other (5–9), the literature contains no information on the use of these techniques to separate the various decomposition products which may occur in barbiturate solutions. For this reason it was felt that a study should be initiated to ascertain if chromatographic procedures could be applied to effect a separation of the decomposed products of the barbiturates. Since the decomposition products of phenobarbital in alkaline solutions are known, this was the barbiturate selected to include in this study.

EXPERIMENTAL

Supplies.—Whatman filter paper No. 1; phenobarbital, U.S.P.; phenobarbital sodium, U.S.P.; phenylethylacetyl urea (prepared in our laboratory); phenylethyl acetic acid, A. R.; *n*-hexyl alcohol, *n*-heptyl alcohol, *n*-octyl alcohol, isopropyl alcohol, methyl alcohol, and ammonium hydroxide 28% (all A. R.); cobalt nitrate solution (1%); bromocresol green solution (0.04% in ethyl alcohol, adjusted to blue-green color with NaOH solution); *p*-dimethylaminobenzaldehyde solution (2% in 1.2 *N* HCl); copper sulfate solution (5% in 2 ml. pyridine and 48 ml. H_2O).

PREPARATION OF SAMPLES

A six per cent phenobarbital sodium solution was placed into ampuls and thermally degraded by storing at 80° for twenty-four hours or more. The ampuls were broken as required and the precipitate collected and dissolved in chloroform. Further extraction of the above aqueous phase with chloroform was then conducted to insure removal of all the chloroform-soluble constituents from the degraded phenobarbital sodium solutions. The chloroform and aqueous fractions were then collected into separate vessels and set aside until needed in the chromatographic studies.

Known decomposition products of phenobarbital sodium were obtained, recrystallized, and separately dissolved in either distilled water or chloroform.[1] At the same time an aqueous solution of phenobarbital sodium and a chloroform solution of phenobarbital were prepared.

Preparation of Chromatogram.—Approximately five microliters of each solution containing a known compound were then spotted on filter paper at equal intervals and at a starting point one and one-half inches from the bottom of the paper. These points appear on all the chromatograms (Figs. 1 and 2) at points A to F. At point G, spots were placed of all the known compounds (A–F) superimposed upon each other. The aqueous and chloroform fractions of the degraded phenobarbital sodium solution were spotted at points H and I, respectively. Finally, a mixture (H and I), spotted separately, of the aqueous and chloroform extracts of the degraded phenobarbital sodium solution was placed at point J.

The paper was then rolled into a cylindrical shape and placed into a battery jar containing a beaker or beakers of the desired solvent system. The lid was placed securely on the jar and the atmosphere in the chamber and the solvent system were allowed to equilibrate overnight, at which time the mobile phase of the solvent system was added to the bottom of the jar. After preliminary experimentation, it was found that eighteen hours were sufficient for the solvent front to reach the desired height. At the end of this period the chromatograms were removed and allowed to dry. It was then viewed in ultraviolet light. Phenobarbital sodium, phenylethylacetyl urea, and

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[1] Approximately 2% w/v solutions.

Fig. 1.—Separation of the decomposition products of phenobarbital sodium by paper chromatography. Solvent system: isopropyl alcohol, 125 ml.; *n*-hexyl, or heptyl, or octyl alcohol, 75 ml.; methanol, 50 ml.; ammonium hydroxide, 50 ml.

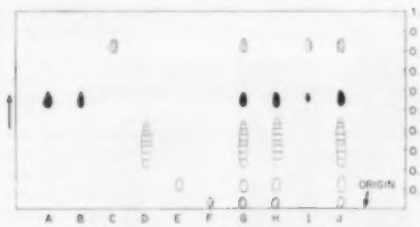

Fig. 2.—Separation of the decomposition products of phenobarbital sodium by paper chromatography. Solvent system: *n*-amyl alcohol saturated with borate buffer, pH 10.

Key to the letters and symbols included in Figs. 1 and 2: A—phenobarbital in chloroform; B—phenobarbital sodium in water; C—phenylethylacetyl urea in chloroform; D—phenylethyl acetic acid in chloroforom; E—urea in water; F—sodium bicarbonate in water; G—mixture of A to F; H—aqueous fraction of decomposed phenobarbital sodium solution; I—chloroform fraction of decomposed phenobarbital sodium solution; J—mixture of H and I; solid area—phenobarbital and phenobarbital sodium; enclosed vertical lines—phenylethylacetyl urea; enclosed horizontal lines—phenylethyl acetic acid; open area—urea; enclosed dotted area—sodium bicarbonate.

phenylethyl acetic acid were evident in the presence of the ultraviolet light. Spraying techniques were also employed to delineate the various compounds on the chromatograms.

Solvent System.—In these studies two types of solvent systems were employed, a two phase and a one phase system.

The two phase solvent system was prepared by shaking the solvents for five minutes in a separatory funnel and then allowing the phases to separate by standing for two hours at room temperature. In this type of system, the following solvent combinations were employed: *n*-hexyl alcohol saturated with ammonium hydroxide (28%); *n*-hexyl alcohol saturated with borate buffer (pH 10); and *n*-amyl alcohol saturated with borate buffer (pH 10).

When the buffers were employed, the filter papers were first sprayed with buffer solution and dried before they were spotted.

For the one phase solvent system, the following combination of solvents was employed:

isopropyl alcohol	125 ml.
n-hexyl alcohol or *n*-heptyl alcohol or *n*-octyl alcohol	75 ml.
methyl alcohol	50 ml.
ammonium hydroxide (28%)	50 ml.

Spraying Reagents.—The barbiturates were identified by spraying with cobalt nitrate solution, drying, and exposing the chromatogram to ammonia vapors. Pink spots developed for the barbiturates. Phenylethyl acetic acid appeared as a bluish-green spot when sprayed with copper sulfate solution. The solution of bromocresol green produced a yellow spot for the acidic compounds and a blue spot for the basic compounds. The barbituric acids were not stained by this reagent because of their weakly acidic properties. Urea was stained as a bright yellow color when sprayed with *p*-dimethylaminobenzaldehyde solution.

The spraying reagents did not stain any of the above compounds except urea when the filter paper was buffered; therefore, ultraviolet light was utilized for visualizing the compounds.

RESULTS

Table I includes the R_f values obtained in the different solvent systems for the identified decomposed products of phenobarbital sodium. Each solvent system is discussed below.

***n*-Hexyl Alcohol Saturated With Ammonium Hydroxide System.**—When this solvent system was employed, it was possible to separate phenylethylacetyl urea, phenylethyl acetic acid, and phenobarbital sodium from the decomposition mixture. Urea was not completely separated from phenobarbital sodium. Basic spots were found at the origin and were later identified as sodium bicarbonate with some trace impurities.

***n*-Hexyl Alcohol Saturated With Borate Buffer System.**—With this solvent system, it was possible to separate phenylethylacetyl urea, phenobarbital sodium, and phenylethyl acetic acid. Urea, however, could not be separated from the phenylethyl acetic acid.

Isopropyl Alcohol-Hexyl Alcohol-Methyl Alcohol-Ammonium Hydroxide System.—In this one phase

TABLE I.—SOLVENT SYSTEM AND R_f VALUES FOR DEGRADATION PRODUCTS OF PHENOBARBITAL SODIUM

Name of Compound	*n*-Hexyl Alcohol Saturated With Ammonium Hydroxide	*n*-Hexyl Alcohol Saturated With Borate Buffer pH 10	Isopropyl Alcohol, *n*-Hexyl, *n*-Heptyl, or *n*-Octyl Alcohol Methyl Alcohol, Ammonium Hydroxide	*n*-Amyl Alcohol Saturated With Borate Buffer pH 10
Phenobarbital sodium	0.17	0.48	0.40	0.55
Phenylethylacetyl urea	0.90	0.85	0.81	0.83
Phenylethyl acetic acid	0.41	0.23	0.52	0.32
Urea	0.09	0.09	0.30	0.13

system (Fig. 1), a separation was achieved of the decomposition products in phenobarbital sodium solutions. Spots for phenylethylacetyl urea, phenylethyl acetic acid, phenobarbital sodium, and urea appear. If the *n*-hexyl alcohol is replaced in the above system with *n*-heptyl or *n*-octyl alcohol no change will occur in R_f values (Table I). However, there appears a "tailing" of phenylethylacetic acid with the heptyl or octyl alcohols. The basic spot at the origin was identified as sodium bicarbonate.

Amyl Alcohol Saturated With Borate Buffer.—A very good separation of the four known decomposition products of phenobarbital (Fig. 2) was obtained.

SUMMARY

1. An ascending paper chromatographic technique was employed to separate the degradation products, reported by Rotondaro, of phenobarbital sodium in aqueous solutions.

2. Two solvent systems were found suitable to effect separation of the four decomposed products of phenobarbital sodium in an aqueous medium. One system employed *n*-amyl alcohol saturated with borate buffer while the second was a one phase system composed of isopropyl alcohol, *n*-hexyl alcohol, methyl alcohol, and ammonium hydroxide.

3. Other degraded barbiturate solutions are being investigated by the method reported in this paper with the hope of separating and identifying the decomposed compounds.

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A New Benzodioxan Derivative With Adrenergic Blocking Activity*

2-(N,N-diallylcarbamylmethyl)aminomethyl-1,4-benzodioxan

By G. VICTOR ROSSI and MARVIN E. ROSENTHAL

The compound 2-(N,N-diallylcarbamylmethyl) aminomethyl-1,4-benzodioxan (A-2275), selected for evaluation from a series of new benzodioxan derivatives, produced moderate but rather transient reduction of blood pressure in anesthetized normotensive dogs and unanesthetized hypertensive rats. Its adrenergic blocking activity was less, and its direct vasoconstrictor effect was greater than that exhibited by piperoxan hydrochloride. Compound A-2275 possessed low acute toxicity and provided moderate protection against the lethal effects of epinephrine in mice.

A PREVIOUS REPORT by Rossi and Smith (1) described the evaluation of the hypotensive and adrenergic blocking activity of a new series of 2-[N-alkyl(N,N-dialkylcarbamylmethylamino) alkyl]-1,4-benzodioxan derivatives. Synthesis and preliminary pharmacologic evaluation of additional members of this series indicated that 2-(N,N-diallylcarbamylmethyl)aminomethyl-1,4-benzodioxan (designated as compound A-2275) possessed significant depressor activity. The pharmacologic investigation of compound A-2275 forms the basis of the present report.

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EXPERIMENTAL

Acute Toxicity in Mice.—Swiss Webster male mice (Huntingdon Farms), weighing from 18 to 22 Gm. and fasted for twelve hours with free access to water, were used in determining the oral, intraperitoneal, and intravenous LD_{50} values of compound A-2275[1] and piperoxan hydrochloride (Benodaine).[2] Following preliminary "range-finding" experiments, each LD_{50} was established from data obtained with five groups of ten mice which received dosages spaced at 0.1 log intervals. Those animals which succumbed, did so within two hours after administration of the benzodioxan derivatives, and in each case death was preceded by hyperexcitability, tremor, clonic and tonic convulsions. LD_{50} values and their 95% con-

[1] Generously supplied by Dr. Gustav J. Martin, National Drug Co. Research Laboratories, Philadelphia, Pa.
[2] Generously supplied by Edmund McLaughlin, Merck Sharp and Dohme, West Point, Pa.

fidence limits, calculated according to the method of Epstein and Churchman (2) are presented in Table I.

TABLE I.—ACUTE TOXICITY OF PIPEROXAN HYDROCHLORIDE AND COMPOUND A-2275 IN MICE

	LD_{50} mg./Kg.	95% Confidence Limits
Piperoxan hydrochloride		
Intravenous	33.2	30.2– 36.4
Intraperitoneal	175.0	159.0–189.0
Oral	502.0	427.0–587.0
Compound A-2275		
Intravenous	55.0	50.2– 60.1
Intraperitoneal	230.0	209.0–252.0
Oral	447.0	392.0–509.0

The data do not demonstrate any outstanding difference in acute toxicity between piperoxan hydrochloride and compound A-2275, however, the ratios between the intravenous and oral LD_{50}'s suggest that the latter compound may be absorbed to a greater extent following oral administration.

Reduction of Epinephrine Toxicity in Mice.—The degree and duration of protection provided by compound A-2275, piperoxan hydrochloride, and tolazoline hydrochloride (Priscoline) were compared in mice (Swiss Webster male, 18–22 Gm., fasted twelve hours) according to the method of Loew and Micetich (3). Varying doses of the three adrenergic blocking agents or 0.5 ml. of distilled water were injected intraperitoneally into a number of groups of ten mice. At intervals of fifteen, thirty, sixty, one hundred and twenty, and two hundred and forty minutes after administration of either distilled water or the test compounds, 14.4 mg. of *l*-epinephrine hydrochloride was injected intraperitoneally into groups of pretreated mice and mortality occurring after one hour was recorded (Table II).

The dose of epinephrine employed in this study killed an average of 86% (N = 50) of the control animals (previously injected intraperitoneally with 0.5 ml. of distilled water). Piperoxan hydrochloride in doses of 50 and 100 mg./Kg. failed to confer protection against the lethal effect of epinephrine. Loew and Micetich (3) similarly reported piperoxan to be noneffective in this respect. Administration of epinephrine fifteen minutes after intraperitoneal injection of 50 mg./Kg. of compound A-2275 resulted in fewer fatalities than in the control group. Maximal protection (50% mortality) occurred within thirty minutes, and after one hour the blocking action had largely terminated. Only slightly greater reduction of epinephrine toxicity was obtained with 100 mg./Kg. of compound A-2275. A considerably greater degree and duration of protection against the toxic effect of epinephrine was provided by significantly smaller doses (10 and 20 mg./Kg.) of tolazoline.

Although the benzodioxan compound A-2275 moderately decreased the toxicity of epinephrine in mice, the doses required to elicit protection were considerably in excess of those producing significant depressor activity as described in subsequent sections of this report.

Hypotensive Activity in Normotensive Dogs.—Compound A-2275 and piperoxan hydrochloride were compared in regard to their ability to lower blood pressure and reduce the pressor response to epinephrine and occlusion of the carotid arteries in mongrel dogs (8 to 10 Kg., both sexes) anesthetized with 35 mg./Kg. of pentobarbital sodium. Blood pressure was recorded directly from a femoral artery. Epinephrine hydrochloride (2 μg./Kg.) was injected intravenously (femoral vein) thirty minutes after administration of the compound being examined. After stabilization of the blood pressure, the carotid sinus reflex was evoked by clamping both common carotid arteries for an interval of thirty seconds. The changes in blood pressure following each procedure were compared with the average of three uniform pressor responses elicited prior to administration of the hypotensive agent. The mean reduction in blood pressure and inhibition of the pressor effect of epinephrine and carotid artery occlusion obtained with each dose level and route of administration are presented in Table III.

Compound A-2275 injected intravenously in a dose of 1 mg./Kg. produced a moderate, rapid but transient (fifteen to thirty minutes duration) reduction in blood pressure and the response to epinephrine and carotid artery occlusion. Increased intravenous doses (as much as 16 mg./Kg.) did not elicit appreciably greater decreases in blood pressure, but the duration of hypotension was increased from sixty to one hundred and eighty minutes. Doses of 32 mg./Kg. intravenously were uniformly fatal. The partial adrenergic blockade produced by even larger doses of compound A-2275 was abolished by increasing the amount of epinephrine injected to 4 or 8 μg./Kg. Similar blockade of the pressor response to norepinephrine (2 μg./Kg.) was observed, however the effects of intravenous isopropylarterenol (2 μg./Kg.) were not significantly altered.

The reduction of blood pressure following oral administration of 25 mg./Kg. of compound A-2275 was maximal within forty-five minutes and was largely terminated after three hours. Increasing the oral dosage to 50 mg./Kg. increased the onset and duration of action (four hours) but did not ap-

TABLE II.—REDUCTION OF EPINEPHRINE TOXICITY IN MICE

Test Compound	mg./Kg. i. p.	Per Cent Mortality[a] Minutes after Administration of Test Compound 15	30	60	120	240
Tolazoline hydrochloride	10	10[b]	20	60	70	90
	20	10	10	40	50	80
Piperoxan hydrochloride	50	80	90	100	90	80
	100	90	100	100	100	90
Compound A-2275	50	60	50	80	80	90
	100	50	50	70	90	100
Distilled water	0.5 ml./mouse	80	100	80	80	90

[a] Deaths recorded one hour after injection of epinephrine hydrochloride—14.4 mg./Kg., i. p. [b] N = 10 in all groups.

TABLE III.—DEPRESSOR ACTIVITY OF COMPOUND A-2275 AND PIPEROXAN IN THE ANESTHETIZED NORMOTENSIVE DOG

Compound	Dose mg./Kg.	Route of Administration	No. Animals	Mean Per Cent Decrease in Initial Blood Pressure	Mean Per Cent Decrease in Pressor Response, 30 min. after Drug	
					Epinephrine[a]	Carotid Sinus Occlusion[b]
A-2275	1	i. v.	5	20	16	34
A-2275	2	i. v.	7	23	18	42
A-2275	4	i. v.	5	26	38	48
A-2275	8	i. v.	5	24	45	52
A-2275	16	i. v.	4	26	62	65
A-2275	25	*per os*	4	28	40	72
A-2275	50	*per os*	5	38	42	80
Piperoxan	0.25	i. v.	1	0	100	0
Piperoxan	1	i. v.	2	0	reversal	26
Piperoxan	2	i. v.	1	16	reversal	85

[a] Epinephrine = 2 μg./Kg. of epinephrine hydrochloride, intravenously.
[b] Carotid sinus occlusion = clamping both common carotid arteries for thirty seconds.
Per cent decrease in pressor response based on an average of three responses prior to administration of compound A-2275 or piperoxan.

TABLE IV.—COMPARATIVE HYPOTENSIVE EFFECT OF COMPOUND A-2275 AND PIPEROXAN IN THE UNANESTHETIZED HYPERTENSIVE RAT

Compound	Dose mg./Kg.	Route of Administration	Mean Pretreatment Systolic Blood Pressure, mm. Hg	Mean Reduction of Pretreatment Blood Pressure, mm. Hg Time After Administration of Drug, Hr.			
				1	2	4	6
A-2275	1	i. p.	218[a]	18	19	8	2
Piperoxan	1	i. p.	222	18	22	11	2
A-2275	2	i. p.	231	23	31	19	3
Piperoxan	2	i. p.	226	15	27	16	0
A-2275	4	*per os*	230	18	20	11	1
Piperoxan	4	*per os*	215	15	20	10	1
A-2275	8	*per os*	207	24	29	14	2
Piperoxan	8	*per os*	212	21	27	14	2

[a] N = 10 in all groups.

preciably enhance the extent of the blood pressure reduction.

That mechanisms other than adrenergic blockade contribute to the hypotensive activity of compound A-2275 was suggested by the partial inhibition of the pressor responses to epinephrine and norepinephrine obtained in the dog with doses of the compound which produced considerable reduction of blood pressure. Piperoxan abolished or reversed the pressor response to epinephrine in concentrations which elicited no significant effect on blood pressure.

Hypotensive Activity in Hypertensive Rats.[3]—Chronic hypertension was experimentally induced in male Sherman rats weighing approximately 130 Gm. according to the technique previously described by Rossi and Packman (4). Systolic blood pressure was determined in the unanesthetized hypertensive rat by the photoelectric tensometer method (5). The hypotensive activity of compound A-2275 in doses of 1 and 2 mg./Kg. injected intraperitoneally, and 4 and 8 mg./Kg. given orally, was compared in each case with piperoxan hydrochloride administered in the same doses.

No measurable change in the blood pressures of ten control animals was detected when measured one, two, four, and six hours after administration of distilled water orally or intraperitoneally. Significant ($P < 0.001$) reduction in systolic pressure, which was largely dissipated after four hours, followed intraperitoneal injection of 1 mg./Kg. of compound A-2275 (Table IV). Increase in dosage to 2 mg./Kg. intraperitoneally resulted in a slight increase in the extent and duration of the depressor response. Comparable effects were elicited by 4 and 8 mg./Kg. of compound A-2275 administered orally, indicating a moderate degree of gastrointestinal absorption. There was no significant difference in the degree or duration of blood pressure reduction produced by equivalent doses of piperoxan hydrochloride and compound A-2275.

Rat Hind Limb Perfusion.—The direct effect of compound A-2275 and piperoxan hydrochloride on peripheral blood vessels was determined by the use of a modification of the rat hind limb preparation described by Burn (6). In the present study, the abdominal aorta of the anesthetized rat was cannulated and the hind limbs perfused with oxygenated Ringer-Locke solution maintained at 37° and 60 mm. Hg pressure. The abdominal vein was cannulated with polyethylene tubing and arranged so that the perfusate collected in a cylindrical graduate. Volume of the perfusate was recorded at five-minute intervals. After the rate of flow became constant, the responsiveness of the preparation was tested by noting the transient decrease in flow rate following injection of 1–2 μg. of epinephrine hydrochloride into a side-arm of the aortic cannula.

Piperoxan hydrochloride introduced into the system in amounts of 50, 100, and 250 μg. had no consistent effect on the rate of perfusion through the

[3] The authors wish to acknowledge the assistance of Dr. Sidney Goldstein during this phase of the investigation.

rat hind limb preparation (N=6). Relatively large doses, 0.5 and 1 mg., produced a slight reduction (0 to 8 and 5 to 17%, respectively) in flow rate. In contrast, compound A-2275 exhibited moderate direct vasoconstrictor activity. The amounts of compound A-2275 injected and the resulting decreases in perfusion rate were as follows: 50 μg.—10 to 12%; 100 μg.—16 to 26%; 250 μg.—32 to 45%; and 0.5 mg.—60 to 100% (N=8).

Direct stimulation of many different smooth muscles is a property common to many benzodioxans. Although the spasmogenic effect of compound A-2275 on vascular muscle would appear to interfere with its hypotensive activity, it is doubtful whether the blood concentrations achieved with effective depressor doses in the intact animal approximate those which were found to cause significant reduction of perfusion rate in the rat hind limb preparation.

SUMMARY

1. The compound 2-(N,N-diallylcarbamylmethyl) aminomethyl-1,4-benzodioxan (A-2275) was selected for pharmacologic evaluation from a series of new benzodioxan derivatives on the basis of hypotensive activity.

2. Oral, intraperitoneal, and intravenous LD_{50} values for compound A-2275 and piperoxan hydrochloride were determined in mice.

3. Compound A-2275, piperoxan hydrochloride, and tolazoline hydrochloride were compared in regard to their ability to provide protection against the lethal effect of epinephrine in mice.

4. Intravenous injection or oral administration of compound A-2275 produced moderate, but rather transient reduction of blood pressure, and partial inhibition of the pressor responses to epinephrine, norepinephrine, and carotid sinus occlusion in anesthetized normotensive dogs. Piperoxan hydrochloride, injected intravenously in subhypotensive doses, completely abolished the pressor responses to epinephrine and norepinephrine.

5. Comparable blood pressure reduction was obtained with equal doses of compound A-2275 and piperoxan hydrochloride administered orally and intraperitoneally to unanesthetized chronic hypertensive rats.

6. Compound A-2275 was found to possess moderate direct vasoconstrictor activity in the rat hind limb perfusion preparation. Piperoxan hydrochloride exhibited measurable spasmogenic activity only in relatively large concentrations.

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The Preparation of Oxidized Derivatives of 1-Ethylsulfonyl-4-ethylpiperazine*

By WILLIAM O. FOYE and LEO R. FEDOR, Jr.†

N-Substituted derivatives of 1-ethylsulfonylpiperazine have been prepared following the announcement of 1-ethylsulfonyl-4-ethylpiperazine as an antishock agent. Derivatives representing possible oxidation products of the N′-ethyl group were prepared by introducing carboxymethyl and hydroxyethyl groups into 1-ethylsulfonylpiperazine. The latter compound was obtained from 1-benzyloxycarbonylpiperazine, and the benzyloxycarbonyl group was removed by catalytic hydrogenation and by nonhydrolytic cleavage without cleavage of the ethylsulfonyl group.

THE EFFECTIVENESS of 1-ethylsulfonyl-4-ethylpiperazine in treating hemorrhagic shock in dogs was reported by Bovet, *et al.* (1), in 1948. Use of the same compound in the treatment of burn shock in rats was reported by Orth, *et al.* (2), in 1954. The latter group observed that the activity of this compound in preventing shock was delayed in warm weather when the metabolic rate of the rat is slower. This suggested that a metabolite was responsible for the antishock effects, and an oxidation product was considered a likely possibility. Attempts were made to prepare oxidized derivatives of the N-ethyl group, namely 1-ethylsulfonyl-4-β-hydroxyethylpiperazine and 1-ethylsulfonyl-4-piperazinoacetic acid.

Direct oxidation of the compound was considered to be impractical after attempts to oxidize 1-ethylpiperazine gave no characterizable

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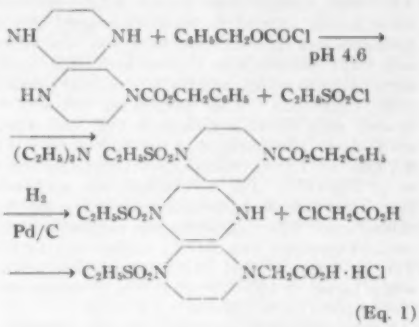
products. Substitution of an already oxidized side-chain on the free nitrogen of 1-ethylsulfonylpiperazine was then considered. The success of this procedure depends, of course, upon having a blocking group present on one of the nitrogens of the piperazine ring, prior to the introduction of the ethylsulfonyl group, which can be removed by a nonhydrolytic procedure. The blocking group most frequently employed for making monosubstituted or unsymmetrical N,N′-disubstituted piperazines has been the carbethoxyl, first introduced by Moore (3) in 1929. Few other easily removable groups have been found which may be monosubstituted in the piperazine ring in suitable yield. Disubstitution is, of course, the rule with piperazine reactions.

The use of 30 per cent hydrogen bromide in glacial acetic acid to cleave the carbethoxyl group from 1-ethylsulfonyl-4-carbethoxypiperazine had been found (4) to give rather poor yields of 1-ethylsulfonylpiperazine, and later attempts gave no better results. The alternative method of synthesis in which the oxidized side-chain is substituted into the piperazine ring first would be complicated by reaction of ethylsulfonyl chloride with the β-hydroxy group or salt of the carboxyl group. Protection of the hydroxyl group would later require a hydrolysis, probably causing elimination of the ethylsulfonyl group as well.

Use of the benzyloxycarbonyl group as a blocking agent was therefore investigated, since it may be cleaved from the piperazine nitrogen by catalytic hydrogenation. 1-Benzyloxycarbonylpiperazine was prepared by a method based on that of Goldman (5), which in turn was based upon the monocarbethoxylation procedure of Moore (3). A fair yield of the monosubstituted piperazine was obtained by conducting the reaction with benzyloxycarbonyl chloride at a controlled pH and separating the disubstituted derivative by extraction with chloroform. 1-Ethylsulfonyl-4-benzyloxycarbonylpiperazine was readily prepared, and both nonhydrolytic cleavage and catalytic hydrogenation procedures were used to remove the benzyloxycarbonyl group. The first-named procedure, using 30 per cent hydrogen bromide in glacial acetic acid, gave a 72 per cent conversion to 1-ethylsulfonylpiperazine hydrobromide, while low pressure hydrogenation over 10 per cent palladium-on-charcoal catalyst gave a 98.5 per cent yield. During the course of this work, Goldman and Williams (6) reported that a series of 1-carbamoyl-4-benzyloxycarbonylpiperazines could also be successfully cleaved by hydrogen and 10 per cent palladium-on-charcoal in refluxing aqueous solution.

The desired 1-ethylsulfonyl-4-β-hydroxyethylpiperazine was obtained by refluxing the 1-ethylsulfonylpiperazine with ethylene chlorohydrin, and 1-ethylsulfonyl-4-piperazinoacetic acid was obtained by heating 1-ethylsulfonylpiperazine with either chloro- or bromoacetic acid. The bromo acid gave a 45.5 per cent yield, while the chloro acid gave 80.5 per cent. Equation 1 illustrates the synthesis of 1-ethylsulfonyl-4-piperazinoacetic acid.



(Eq. 1)

It was also decided to prepare 1-β-hydroxyethylpiperazine and 1-piperazinoacetic acid for comparative purposes, and also because no biological testing has been reported for these compounds. The first named compound was prepared as the dihydrochloride according to the procedure of Moore (3), using 1-carbethoxypiperazine, ethylene chlorohydrin, and sodium carbonate, followed by aqueous hydrolysis of the carbethoxyl group. 1-Piperazinoacetic acid was obtained as the dihydrobromide from 1-carbethoxypiperazine and bromoacetic acid, followed by hydrogen bromide cleavage. Moore (3) obtained this compound by a much more involved procedure from 1-carbethoxypiperazine requiring a lengthy acid hydrolysis, neutralization with silver carbonate, and removal of excess silver.

Antishock tests have not yet been carried out with these compounds. However, four of the compounds, 1-piperazinoacetic acid dihydrobromide, 1-β-hydroxyethylpiperazine dihydrochloride, 1-ethylsulfonyl-4-piperazinoacetic acid hydrochloride, and 1-ethylsulfonyl-4-β-hydroxyethylpiperazine hydrochloride were tested against several parasitic infections in mice at the Lilly Research Laboratories. All of the compounds were ineffective against *T. equiperdum*, *S. novyi*, *S. obvelata*, and *A. tetraptera*, but 1-ethylsulfonyl-4-piperazinoacetic acid hydrochloride was effective against *E. histolytica* at a concentration of 62.5 μg./ml. *in vitro*.

EXPERIMENTAL

Melting points were taken on a Fisher-Johns block and are uncorrected. Analyses were obtained from the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

1-Benzyloxycarbonylpiperazine.—A solution of 30 Gm. (0.155 mole) of piperazine hexahydrate, 225 ml. of methanol, 30 ml. of water, and 46.5 ml. of 6 *N* hydrochloric acid was heated to boiling, and 29.3 Gm. (0.172 mole) of benzyloxycarbonyl chloride was added over a one-half hour period along with sufficient 4 *N* sodium hydroxide to maintain the pH near 4.6. The mixture was heated for an additional three hours, allowed to stand overnight, and the methanol was then removed *in vacuo*. The residue was made alkaline with sodium hydroxide, sodium carbonate was added, and the mixture was extracted with chloroform. The chloroform extract was treated with dilute hydrochloric acid and washed with water. Evaporation of the chloroform yielded 5.5 Gm. of 1,4-bis-(benzyloxycarbonyl)-piperazine, m. p. 112–113°. The acid extract was made alkaline with sodium hydroxide and extracted with chloroform. The chloroform was evaporated under reduced pressure, and the oily residue was distilled. The yield was 14 Gm. (41%) of colorless product which boiled at 160–170° (2–3 mm.); the recorded boiling point (5) is 158–161° (1.4 mm.).

1-Ethylsulfonyl-4-benzyloxycarbonylpiperazine.—A solution of 13.95 Gm. (0.063 mole) of 1-benzyloxycarbonylpiperazine, 100 ml. of anhydrous ether, and 6.5 Gm. (0.064 mole) of triethylamine was treated with an ether solution of 8.2 Gm. (0.064 mole) of ethylsulfonyl chloride drop by drop. The reaction temperature was kept at 15–20° by water cooling. After the addition was complete, the reaction mixture was stirred for two hours at room temperature. A precipitate of triethylamine hydrochloride was filtered from the solution, and white platelets then crystallized from the filtrate after partial evaporation. Soxhlet extraction of the triethylamine hydrochloride with ether yielded an additional quantity of this material. A total of 15.2 Gm. (76.8%) of 1-ethylsulfonyl-4-benzyloxycarbonylpiperazine was obtained; m. p. 108–110°.

Anal.—Calcd. for $C_{14}H_{20}N_2O_4S$: C, 53.85; H, 6.41. Found: C, 53.87; H, 6.36.

1-Ethylsulfonylpiperazine Hydrobromide by Nonhydrolytic Cleavage.—A solution of 1-ethylsulfonyl-4-benzyloxycarbonylpiperazine (3.4 Gm., 0.011 mole), glacial acetic acid (25 ml.), and 30% hydrogen bromide in glacial acetic acid (5 ml.) (Eastman Organic Chemicals) was allowed to stand at room temperature for fifteen minutes and in the refrigerator overnight. The reaction was then warmed to room temperature, ether was added, and the precipitate was collected. Recrystallization from ethanol yielded 2 Gm. (72%) of 1-ethylsulfonylpiperazine hydrobromide; m. p. 218–220°. The recorded m. p. (4) is 216–217.5°.

1-Ethylsulfonylpiperazine by Catalytic Hydrogenation.—In a pressure bottle was placed 9.6 Gm. (0.031 mole) of 1-ethylsulfonyl-4-benzyloxycarbonylpiperazine in 100 ml. of ethanol. The mixture was warmed to 45°, 2 Gm. of 10% palladium-on-charcoal was added, and the mixture was hydrogenated for one-half hour at three atmospheres pressure. The catalyst was then removed by filtration, and the solvent was evaporated under reduced pressure. A yield of 5.4 Gm. (98.5%) of 1-ethylsulfonylpiperazine was obtained which boiled at 145–150° (2 mm.). The hydrobromide melted at 217–219°, which agrees with the reported value (4).

1-Ethylsulfonyl-4-β-hydroxyethylpiperazine Hydrochloride.—A solution containing 1.2 Gm. (0.007 mole) of 1-ethylsulfonylpiperazine and 5 Gm. (0.062 mole) of ethylene chlorohydrin was refluxed for five hours. The excess ethylene chlorohydrin was evaporated, and the resulting oil was crystallized from ethanol. A yield of 0.5 Gm. (29%) of crystalline solid was obtained which melted at 167–168°.

Anal.—Calcd. for $C_8H_{19}N_2O_3SCl$: C, 37.33; H, 7.35. Found: C, 37.38; H, 7.41.

1-Ethylsulfonyl-4-piperazinoacetic Acid Hydrobromide.—A mixture of 1-ethylsulfonylpiperazine (0.5 Gm., 0.003 mole) and bromoacetic acid (0.4 Gm., 0.003 mole) was heated on a steam bath for fifteen minutes. An oil was obtained which was crystallized with difficulty from ethanol; m. p. 176–177°, with previous softening. The yield was 0.4 Gm. or 45.5%.

Anal.—Calcd. for $C_8H_{17}N_2O_4SBr$: C, 30.28; H, 5.36. Found: C, 29.98; H, 5.77.

1-Ethylsulfonyl-4-piperazinoacetic Acid Hydrochloride.—A solution of 1-ethylsulfonylpiperazine (0.5 Gm., 0.003 mole) and chloroacetic acid (0.26 Gm., 0.003 mole) in 25 ml. of chloroform was refluxed for one hour. The chloroform was evaporated, and the residue was crystallized from ethanol. The yield was 0.62 Gm. (80.5%) of product which melted at 132–133°.

Anal.—Calcd. for $C_8H_{17}N_2O_4SCl$: C, 35.20; H, 6.24. Found: C, 35.28; H, 6.14.

1-Carbethoxy-4-β-hydroxyethylpiperazine.—This compound was prepared by the procedure of Moore, Boyle, and Thorn (3). A 66% yield of colorless liquid was obtained; b. p. 190–192° (20 mm.) (lit. (3) b. p. 184° (17 mm.)).

1-β-Hydroxyethylpiperazine Dihydrochloride.—This product was obtained by the procedure of Moore, Boyle, and Thorn (3). A granular white solid was obtained which melted at 192–193° with previous softening. No melting point has been reported for this compound.

Anal.—Calcd. for $C_6H_{16}N_2OCl_2$: Cl, 34.9%. Found: Cl, 33.9%.

1-Piperazinoacetic Acid Dihydrobromide.—A mixture of 7 Gm. (0.044 mole) of 1-carbethoxypiperazine (3) and 6.2 Gm. (0.044 mole) of bromoacetic acid was heated on a steam bath for one-half hour. A glassy semisolid was obtained which was treated with 5 ml. of 30% hydrogen bromide in glacial acetic acid. The solution was heated at 75° for one-half hour, and after cooling, 3.5 Gm. (30%) of white solid was obtained which did not melt under 300°.

Anal.—Calcd. for $C_6H_{14}N_2O_2Br_2$: N, 9.15. Found N, 9.18.

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Tritium-Labeled Meprobamate*

By L. J. ROTH†, K. E. WILZBACH‡, A. HELLER†, and L. KAPLAN‡

Meprobamate has been labeled with tritium by exposure to tritium gas. Its radiochemical purity has been convincingly demonstrated, and the distribution of tritium within the molecule has been determined. The specific activity of 300 mc. per Gm. is the highest thus far reported for a product labeled by this technique.

THE SYNTHESIS of 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate (meprobamate) was described by Ludwig and Piech (1). The pharmacological properties of this tranquilizing drug have been described by Berger (2). Studies of its transport, metabolism, and cellular localization in the central nervous system are important and can be facilitated by use of a radioactive labeled compound. Because of the advantages of resolution which obtain with tritium in autoradiography, it was desirable to prepare radiochemically pure tritium-labeled meprobamate of high specific activity.

Labeled meprobamate with a specific activity of 50 μc. per Gm. has been prepared by the recoil triton method (3). Significantly higher activities are precluded in this method by the concomitant decomposition which occurs. Agranoff, Bradley, and Axelrod (4) have reported the labeling of meprobamate by exposure to tritium gas as described by Wilzbach (5), but do not mention the specific activity attained. We have now made a more detailed study of the labeling of meprobamate by this method in order to establish unequivocally the radiochemical purity of the product and to obtain information, needed for the interpretation of metabolic studies, of the distribution of the isotope within the molecule.

PROCEDURES

Tritium Assay.—Tritium was determined by ion current measurement (6) on gas samples prepared by reductive decomposition of organic compounds with zinc and nickel oxide (7).

Paper chromatograms were scanned for tritium using the apparatus described by Kisieleski and Smetana (8).

Tritiation.—Meprobamate, obtained from Wallace Laboratories, New Brunswick, N. J., was recrystallized from water (m. p. 105°, literature 105–106°) (1). One gram of the compound was exposed for seventy-two hours to 5.5 curies of tritium gas (4.17 cc. S. T. P., 26.4% He, 8.5% H, 65.1% T). The tritium gas was removed and the product was treated twice with 50-ml. portions of methanol to remove labile tritium. Sixty millicuries of tritium was found in the methanol; the nonvolatile residue contained 350 mc.

Purification.—The crude meprobamate was recrystallized once from water, then dissolved in methanol and treated with Norite, and subsequently subjected to a series of recrystallizations from water and benzene. The specific activity of the product after each recrystallization is shown in Table I. It is seen that after treatment with decolorizing carbon the product is essentially radiochemically pure. The recovery of material at this point was 88%.

Paper chromatography of the purified meprobamate with toluene-*n*-butanol-H_2O (19:1:20, upper phase) showed only a single radioactive peak with an R_f value of 0.35 (Fig. 1). Biological stability of the label was established by i.p. injection in a rat. Unmetabolized meprobamate recovered from the urine by recrystallization had the same specific activity (±2%) as the jnjected dose.

TABLE I.—PURIFICATION OF MEPROBAMATE-T BY SUCCESSIVE RECRYSTALLIZATION

Solvent	Activity, μc./mg.
Water	344
Methanol + Norite	[a]
Water	305
Water	303
Benzene	304
Benzene	302
Water	303

[a] Not determined.

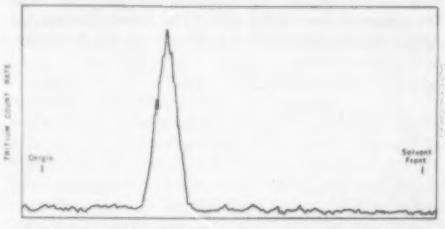

Fig. 1.—Radiometric scan of paper chromatogram of meprobamate T. Solvent: toluene, *n*-butanol, water (19:1:20).

Preparation of Derivatives.—For the preparation of derivatives, 1.15 mg. of the purified meprobamate-T was diluted with 2.913 Gm. of the inactive compound and recrystallized from water and benzene. The resultant product had a specific activity of 0.122 μc./mg. (26.6 μc./mole). The following derivatives were prepared and assayed (Table II):

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‡ Argonne National Laboratory.
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TABLE II.—DERIVATIVES OF MEPROBAMATE-T

Compound	Activity	
	μc./mg.	μc./mole
Meprobamate	0.122	26.6
Diacetyl-1	0.089	26.9
Dixanthyl-2	0.0461	26.7
Diol-3	0.200	26.4
Diacid		
Acid Oxidation-4	0.109	17.5
Alkaline Oxidation-5	0.111	17.8

1. **N,N′-Diacetyl-2-methyl-2-n-propyl-1,3-propanediol Dicarbamate.**—This was prepared by acetylation with acetic anhydride and sulfuric acid and recrystallized from absolute alcohol, m. p. 134–135° (9).

2. **N,N′-Dixanthyl-2-methyl-2-n-propyl-1,3-propanediol Dicarbamate.**—This was prepared by heating on a steam bath for thirty minutes a solution of meprobamate and twice the calculated quantity of xanthydrol in a 1:1 mixture of glacial acetic acid and absolute ethanol. Recrystallization from 95% alcohol gave a product, m. p. 182° (9).

3. **2-Methyl-2-n-propyl-1,3-propanediol.**—Meprobamate was hydrolyzed with alcoholic KOH. The product was extracted with ether and recrystallized from hexane, m. p. 56°, Lit. 62–63° (10).

4. **Methyl-n-propylmalonic Acid**—*Acid Oxidation.*—Cold fuming nitric acid (sp. gr. 1.50) was cautiously added to 300 mg. of the 2-methyl-2-*n*-propyl propanediol in a small flask chilled in an ice bath until the diol was completely dissolved. The flask was stoppered loosely and allowed to stand for two days at room temperature. The mixture was made alkaline with KOH and washed twice with ether. The solution was then acidified to pH 2 and extracted twice with ether. The extracts were washed with water and dried over sodium sulfate. The methyl-*n*-propylmalonic acid, obtained by evaporation of the extract, was recrystallized from benzene, m. p. 106°.

5. **Methyl-n-propylmalonic Acid**—*Alkaline Oxidation.*—To 300 mg. of the diol dissolved in 15 ml. of water were added 1.0 Gm. of potassium permanganate in 70 ml. of water and 0.9 Gm. of anhydrous sodium carbonate. This mixture was shaken thoroughly, stoppered, and allowed to stand at room temperature for one week. The excess permanganate was destroyed by addition of 0.1*N* sodium bisulfite, the mixture was filtered, and the product was isolated and purified as above, m. p. 106°.

DISCUSSION

The recrystallization to constant specific activity (Table I), the identity of the molar activities of the derivatives with that of the meprobamate (Table II), and the presence of a single radiochemical peak in paper chromatography (Fig. 1) are convincing evidence for the radiochemical purity of the product. The specific activity of 303 mc. per Gm. (one atom of tritium per 440 molecules of meprobamate) is the highest yet reported for this method. This value corresponds to an incorporation rate of 18 mc. of tritium per curie-day exposure, or a G value of 2.1 atoms of tritium per 100 electron volts. The appearance of 74% of the total tritium incorporated from the gas, and 86% of the stably-bound tritium in meprobamate is unusually high for this method. The material obtained is of sufficiently high specific activity for use in autoradiography, and results of such studies will be published at a later date. The ease of purification and the high recovery of radiochemically pure material indicate that substantially higher specific activities could be attained if desired.

Upon oxidation of the diol to the corresponding malonic acid, the loss of tritium from the hydroxymethylene groups is 33.8% (Table II). The ratio of tritium to hydrogen in these methylene groups is thus 1.28 times the average value for the alkyl substituents. The fact that tritium is rather randomly distributed in the molecule should make it possible to detect any of the products resulting from metabolic changes in the diol moiety of meprobamate, since the label was also demonstrated to be biologically stable.

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A Chromatographic Examination of the Alkaloidal Fraction of *Amanita pantherina**

By L. R. BRADY and V. E. TYLER, Jr.

A 1.5-Kg. quantity of oven-dried *Amanita pantherina* was defatted and extracted exhaustively with 70% ethanol. Purification of this extract yielded a solution which contained the total chloroform-soluble alkaloids of the mushroom. Chromatographic examination of this solution revealed the presence of two compounds giving typical alkaloid reactions and a third compound which appeared to be an indole derivative. Comparison of R_f values and color reactions of these compounds with known alkaloids revealed that neither bufotenine nor hyoscyamine, nor any alkaloid giving a positive Vitali-Morin reaction, was present in the extract.

THE PANTHER fungus, *Amanita pantherina* (Fr.) Quélet, grows abundantly in western Washington where it is responsible for a number of cases of mushroom poisoning annually. Collected in the button stage before the universal veil ruptures, the stipe lengthens and the pileus expands, it is commonly confused with puffballs and even truffles by the layman. Persons who have ingested it exhibit symptoms which are not entirely typical of muscarine poisoning in that they experience vivid hallucinations of the type induced by certain tropane (hyoscyamine, scopolamine) or indole (bufotenine, N,N-dimethyltryptamine) alkaloids (1).

Both of these types of alkaloids have been reported to occur in *A. pantherina*. In 1953, Wieland and Motzel (2) claimed to have detected, by chromatographic means, the presence of bufotenine in *A. pantherina* as well as in *Amanita muscaria* (Fr.) S. F. Gray. These investigators did not publish experimental details of this work, and the presence of the compound has not been confirmed by others.

The literature pertaining to pilzatropine, the hypothetical alkaloid of *A. pantherina* and *A. muscaria*, has been reviewed recently (3). Lewis (4) reported in 1955 the isolation of *l*-hyoscyamine from small samples of these two species, apparently confirming the claims of some previous investigators who reported the separation of alkaloids with atropine-like activity from the muscarine fraction of these mushrooms. However, a year prior to Lewis's report, Kwasniewski (5) had been unable to establish the presence of a base with mydriatic activity in much larger quantities of *A. muscaria*.

Due to the difficulties involved in collecting a sufficient quantity of the mushrooms to allow the application of classical analytical procedures, it was decided to employ existing paper chromatographic methods suitable for the identification of extremely small quantities of the tropane and indole alkaloids.

EXPERIMENTAL

Preparation of the Alkaloidal Extract.—A quantity of *A. pantherina* was collected during the winter and spring of 1957–1958 in the vicinity of Olympia, Washington. Several of the collections were made in areas where people had previously obtained panther fungus which, when eaten, produced typical hallucinations. The individual specimens were carefully cleaned, sliced, and dried in a forced-air drying oven at 48° for three to five days. One hundred and sixty-five pounds of fresh mushrooms yielded seven pounds of oven-dried material.

The dried fungus was ground to a coarse powder in a Wiley mill, and a 1.5-Kg. quantity was moistened with petroleum ether and packed in a large percolator. Following a maceration period, the drug was extracted with petroleum ether which was allowed to percolate at a moderate rate until 7.35 L. were collected.

After defatting, the marc was removed from the percolator and allowed to air dry. It was then moistened with 70% ethanol, repacked in the percolator, allowed to macerate for twenty hours, and extracted at a moderate rate with 70% ethanol until 14 L. were collected. This ethanolic extract was concentrated to a thick syrupy liquid at 60° under reduced pressure in a Flash-Evaporator.

The residue was taken up in 2 L. of 1% hydrochloric acid, insoluble resinous matter removed by filtration, and the acid filtrate was shaken out with four successive 200-ml. portions of chloroform to remove traces of fats and other nonalkaloidal ballast materials. After separation of the last portion of chloroform, the aqueous solution was rendered distinctly alkaline with ammonium hydroxide and extracted with five successive 200-ml. portions of chloroform. The combined chloroform extracts were evaporated to a 10-ml. volume at 45° under reduced pressure in a Flash-Evaporator.

One half (5 ml.) of this crude alkaloidal extract was purified further by placing it in a separatory funnel and extracting with three successive portions (5, 3, and 2 ml.) of 2% hydrochloric acid. The

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combined acid extract was then made alkaline with ammonium hydroxide and extracted with three successive portions (2, 2, and 1 ml.) of chloroform. The combined solution which would contain any chloroform-soluble alkaloids originally present in the mushrooms, including the bufotenine and *l*-hyoscyamine reported by previous investigators, was subjected to chromatographic analysis. Each ml. represented 150 Gm. of oven-dried plant material or approximately 3.5 Kg. of fresh material.

Chromatographic Procedures.—Replicate 100-μl. quantities of the alkaloidal extract prepared as described above, (equivalent to 350 Gm. fresh plant material) together with known samples of hyoscyamine, scopolamine, and bufotenine were spotted in successive 10-μl. portions on sheets of Whatman No. 1 filter paper. The reference alkaloids were applied to the sheets singly and as mixtures with themselves and with the extract of the plant material. No appreciable differences in the R_f values of the alkaloids were observed under these different conditions. The sheets were then subjected to ascending formation with a wash liquid composed of the upper phase of a mixture of *n*-butanol-glacial acetic acid-water (4:1:5). After a period of approximately eighteen to twenty hours, they were removed from the chromatographic chamber, dried, and treated with various reagents. These included: (*a*) Thies and Reuther's modification of the Dragendorff reagent (6), a general reagent forming orange-colored spots with most alkaloids; (*b*) 1% cinnamic aldehyde in methanol, followed by exposure to hydrochloric acid vapor (7), giving orange to pink spots with many indole derivatives; (*c*) 2% *p*-dimethylaminobenzaldehyde in 1.2 *N* hydrochloric acid (8), which forms blue to purple-colored spots with many indole derivatives; (*d*) Pauly's reagent (9), which reacts to form orange or pinkish spots with compounds containing a phenolic hydroxy group or with imidazoles.

After spraying with these reagents, three distinct spots were observed on the chromatogram of the purified alkaloidal extract of *A. pantherina*, but none of them corresponded exactly in R_f values or in color reactions to hyoscyamine, scopolamine, or bufotenine. See Table I.

Two spots, apparently alkaloidal in character, were observed which exhibited R_f values in the general region of scopolamine and hyoscyamine. It was decided to test these spots to determine if they represented alkaloids of the solanaceous type. This was carried out by spotting 150-μl. quantities of the purified alkaloidal extract on paper and chromatographing as described. The areas representing the two alkaloidal spots were cut from the paper and eluted with ethanol. After evaporation of the ethanol, the Vitali-Morin test was carried out on the residues as described by Cromwell (10).

Two additional reagents were employed in an attempt to learn something about the nature of the compounds which gave the positive tests for alkaloids. Strips were treated with a sodium nitroprusside-acetaldehyde reagent (11) which gives positive reactions with secondary aliphatic and alicyclic amines and with König's reagent (12) which is useful for detecting pyridine derivatives.

Since the R_f values of a number of indole derivatives are quite similar in the butanol-acetic acid-water system, it appeared worth while to examine the purified alkaloidal extract in a system better suited for the separation of these compounds. A *n*-propanol-1 *N* ammonia (4:1) system was consequently employed for the examination of the extract in the same manner described for the previous solvent system. R_f values and color reactions obtained with this wash liquid are recorded in Table I.

DISCUSSION

The extract of *A. pantherina*, containing the total chloroform-soluble alkaloids of that plant, was subjected to chromatographic examination in a butanol-acetic acid-water system. Two spots were obtained which gave typical alkaloidal reactions with Thies and Reuther's reagent. In addition, a third somewhat diffuse spot was present which gave reactions typical of the indole nucleus with *p*-dimethylaminobenzaldehyde as well as a positive reaction with Pauly's reagent, possibly indicative of a phenolic hydroxy group. No positive reactions were observed when the strips were treated with the sodium nitroprusside-acetaldehyde or with the König's reagent, and it was concluded that the unknown compounds were not secondary amines or derivatives of pyridine.

When subjected to analysis in a *n*-propanol-1 *N* ammonia system, the compounds giving positive Thies and Reuther's reactions apparently failed to

TABLE I.—COLOR REACTIONS AND R_f VALUES OF COMPOUNDS IN ALKALOID FRACTION OF *Amanita pantherina* AND OF REFERENCE COMPOUNDS

Identity of Material	Av. R_f of Spot	Color Reaction with Reagent: Thies and Reuther's	Cinnamic Aldehyde + HCl	*p*-Dimethylaminobenzaldehyde	Pauly's
n-Butanol-Acetic Acid-Water (4:1:5)					
Purified alkaloidal extract	0.87	none	purple	blue	pink
	0.68	orange	none	none	none
	0.51	orange	none	none	none
Hyoscyamine	0.64	orange	none	none	none
Scopolamine	0.55	orange	none	none	none
Bufotenine	0.41	orange	orange	purple	orange-pink
n-Propanol-1 *N* Ammonia (4:1)					
Purified alkaloidal extract	0.86	orange	none	none	none
	0.83	none	purple	blue	pink
Hyoscyamine	0.85	orange	none	none	none
Scopolamine	0.80	orange	none	none	none
Bufotenine	0.75	orange	orange	purple	orange-pink

separate since only one such spot was observed. The indole derivative again appeared as a single spot and still gave a positive Pauly's reaction.

The R_f values and color reactions of the spots in both chromatographic systems failed to correspond exactly with those observed for hyoscyamine and bufotenine, the alkaloids previously reported to exist in this mushroom. The spots which gave typical alkaloid reactions in the butanol-acetic acid-water system and which possessed R_f values quite similar to hyoscyamine and scopolamine, were eluted and subjected to the Vitali-Morin test for solanaceous alkaloids. In spite of the sensitivity of this reaction which will detect as little as 0.0001 mg. of hyoscyamine or scopolamine, the tests were completely negative, no purple color being observed.

Two unidentified compounds possessing general alkaloidal solubilities and giving typical alkaloidal reactions were shown to be present in trace quantities in the sample of *A. pantherina* investigated. A third compound, giving positive reactions for the indole nucleus as well as a positive Pauly's reaction was present in the alkaloid fraction. The latter constituent failed to give a typical alkaloidal reaction with Thies and Reuther's reagent, and the purple color which it exhibited with cinnamic aldehyde was not typical of simple indole derivatives. On the basis of these observations, it was concluded that neither bufotenine nor hyoscyamine, nor any alkaloid giving a positive Vitali-Morin reaction and which might be designated "pilzatropine," was present in the alkaloidal extract. These results are in disagreement with those of Lewis and Wieland and Motzel. Although it is possible that the differences may be due to the existence of strains of *A. pantherina* possessing different biosynthetic abilities, this remains to be determined by the analysis of mushrooms collected in different localities but subjected to uniform analytical procedures.

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Comparative Activities and Toxicities III*

D-Glucamine and Glycine Derivatives of Nitro and Halogenated Benzenes

By EDWARD G. FELDMANN and WILLIAM O. FOYE

D-Glucamine and glycine molecules have been introduced into several nitro and halogenated benzene derivatives in an attempt to reduce the toxic effects to the host of such compounds. A comparison of the acute toxicities of the two types of derivatives showed that the sugar group exerted a definite detoxifying effect, whereas the glycine and glycine ester groups had little effect on toxicity. The antibiotic activities of *m*-dinitrobenzene, 2,4-dinitroaniline, and *p*-nitroaniline were wholly removed when the D-glucamine group was present in these molecules, but N-(2-nitro-4,5-dichlorophenyl)-D-glucamine was found to have a relatively broad antibiotic spectrum. The *o*-, *m*-, and *p*-nitrophenylglycines and their esters revealed a rather limited activity against microorganisms.

MICROBIOLOGICAL INHIBITIONS by aromatic nitro compounds and halides have become a subject for serious investigation since the successful introduction of Chloromycetin and the nitrofurans as antibacterial agents. Examples of this type of compound that have been studied are nitrobenzoic acid derivatives (1), nitroquinolines (2), N-arylglycines (3), and a series of simple nitrobenzene derivatives including, in particular, *m*-dinitrobenzene (4). The metabolism of various halogenated nitrobenzenes has also been investigated (5). Since the toxicity of these aromatic nitro and halogenated compounds to the host is often considerable, an attempt has been made to lower the toxicity of simple aromatic nitro and halogenated compounds by the inclusion of sugar and amino acid residues in these molecules, and perhaps provide a more useful compound of this type. This report describes the results of this attempt. Previously, sugar and amino acid deriva-

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TABLE I.—ACUTE INTRAPERITONEAL TOXICITIES OF NITRO AND HALOGENATED BENZENE DERIVATIVES[a]

No.	Compound	Dose in Mice, mg./Kg.	No. Died[b]/ No. Used	Toxic Signs
1.	*m*-Dinitrobenzene	200	1/5	Death in 2 hr.
		400	4/5	
2.	2,4-Dinitroaniline	200	0/5	Death in 24 hr.
		400	5/5	
3.	N-(2,4-Dinitrophenyl)-D-glucamine	800	0/5	
4.	*p*-Nitroaniline	400	0/5	Death in 2 hr.
		800	5/5	
5.	N-(4-Nitrophenyl)-D-glucamine	800	0/5	None
6.	*p*-Bromobenzamide	400	2/5	Deaths 24–48 hr.
		800	3/5	Deaths in 4 hr.
7.	N-(4-Bromobenzoyl)-D-glucamine	800	0/5	Lethargy and ataxia
8.	N-(2-Nitro-4,5-dichlorophenyl)-D-glucamine	400	0/5	Deaths delayed 24 hr.
		800	5/5	

[a] Determined at the Lilly Research Laboratories by R. C. Anderson.
[b] Observations were made for one week after single injections of compound.

tives of amino- and nitrosalicylic acids (6) and bis-(4-aminophenyl)-sulfone (7) were prepared for the same purpose.

The detoxifying effect of a sugar residue may be seen in Table I, where the acute i.p. toxicities in mice are shown for a series of nitro and halogenated benzenes and their D-glucamine derivatives. A consistent lowering of toxicity was observed when the glucose residue was included in the *p*-nitroaniline, 2,4-dinitroaniline, and *p*-bromobenzamide molecules. Although a limited number of examples is presented, the principle of detoxication by introduction of a sugar molecule into a toxic compound seems to be satisfactorily illustrated. Previous studies, already referred to (6, 7), also showed a consistent lowering of acute toxicity by introduction of sugars into fairly toxic molecules.

The detoxifying effect of an amino acid residue on the nitroaniline molecule was observed after preparation of a series of N-nitrophenyl glycines and their ethyl esters. As may be seen in Table II, no appreciable detoxifying effect on the *p*-nitroaniline molecule was exerted by inclusion of either the glycine or glycine ester residue. Although i.p. toxicities were not determined for *o*- and *m*-nitroaniline, it is unlikely that they would differ a great deal from that of *p*-nitroaniline.

The results of antimicrobial screening (agar dilution technique) of the nitrobenzenes and their D-glucamine derivatives are shown in Table III. A definite narrowing of the antibiotic spectrum is evident when *m*-dinitrobenzene is converted to 2,4-dinitroaniline, and all activity disappeared on further conversion to N-(2,4-dinitrophenyl)-D-glucamine, when testing levels up to 200 μg./ml. were used. Similarly, no activity was shown by N-(4-nitrophenyl)-D-glucamine, and *p*-bromobenzamide and its D-glucamine derivative were also relatively inactive. However, N-(2-nitro-4,5-dichlorophenyl)-D-glucamine was found to have a relatively good antibiotic spectrum, and it showed an appreciable activity against mycobacteria. This activity did not remain when the compound was tested against tuberculosis in mice, unfortunately. The acute i.p. toxicity of this derivative was not much greater than that of the other nitrophenyl-D-glucamine derivatives tested. Antiviral tests were also carried out with the 4-nitrophenyl, 2,4-dinitrophenyl, 4-bromobenzoyl, and 2-nitro-4,5-dichlorophenyl D-glucamines, and all four compounds were ineffective against two types of virus in mice.

TABLE II.—ACUTE INTRAPERITONEAL TOXICITIES OF NITROPHENYL GLYCINES[a]

No.	Compound	LD_{50} + S. E. (in Mice), mg./Kg.
9.	N-(*o*-Nitrophenyl)-glycine	469.9 ± 45.1
10.	N-(*m*-Nitrophenyl)-glycine	739.1 ± 226.1
11.	N-(*p*-Nitrophenyl)-glycine	631.7 ± 106.5
12.	Ethyl N-(*o*-nitrophenyl)-glycinate	500.0 ± 57.0
13.	Ethyl N-(*m*-nitrophenyl)-glycinate	524.8 ± 84.3
14.	Ethyl N-(*p*-nitrophenyl)-glycinate	632.4 ± 66.4

[a] Determined at the Lilly Research Laboratories by R. C. Anderson.

Antimicrobial screening of the N-(nitroaryl)-glycines and their esters revealed a rather limited activity against microorganisms. This is evident from Table IV, where only test organisms which were inhibited are listed. The esters in general showed a somewhat broader spectrum than the free acids, although in neither case did the breadth of activity approach that of the most successful D-glucamine derivative. It is interesting to note that N-(*m*-nitrophenyl)-glycine showed no activity whatsoever, and it has also been shown that the *m*-nitrophenyl halides are metabolized in a different manner than the *o*- and *p*-isomers (5). Some other sugar derivatives, prepared for a pre-

TABLE III.—ANTIBIOTIC SPECTRA OF NITRO AND HALOGENATED BENZENE DERIVATIVES[a]

Test Organism	Inhibitory Concentration μg./ml.[b] Compd. No. 1	2	6	8
Staphylococcus aureus	...	...	...	50
Staphylococcus albus	...	...	...	50
Bacillus subtilis	200	200	...	6.25
Mycobacterium phlei	200	...	...	0.2
Mycobacterium tuberculosis	200	200	...	0.2
Mycobacterium avium	200	200	...	3.13
Escherichia coli	...	...	...	200
Proteus vulgaris	200	...	...	100
Pseudomonas aeruginosa	...	...	...	200
Aerobacter aerogenes	...	...	...	>200
Klebsiella pneumoniae	...	...	...	200
Salmonella enteritidis	...	...	...	>200
Shigella paradysenteriae	50	...	...	25
Saccharomyces pastorianus	100	...	...	25
Candida albicans	50	...	...	100
Trichophyton rubrum	50	200	...	12.5
Trichophyton interdigitale	200	200	...	12.5
Brucella bronchiseptica	...	200	...	200
Vibrio metschnikovii	...	200	...	50
Erwinia amylovora	200	200	...	200
Agrobacterium tumefaciens	100	200	...	200
Xanthomonas campestris	200	200	...	200
Xanthomonas malvacearum	[c]	[c]	...	200
Xanthomonas phaseoli	[c]	[c]	...	200
Pseudomonas solanacearum	50	[c]	...	200
Pseudomonas syringae	>200	...	...	>200
Corynebacterium insidiosum	100	...	...	200
Corynebacterium sepodonicum	100	[c]	...	100
Aspergillus niger	10	200	200	50
Ustilago avenae	100	[c]	...	50
Alternaria solani	[c]	[c]	...	50
Ceratostomella fimbriata	100	...	200	50
Fusarium moniliforme	>200	...	...	50
Fusarium oxysporum lycopersici	200	200	...	200
Colletotrichum gossypii	10	[c]	...	50
Glomerella singulata	50	...	...	50
Verticillium albo-atrum	100	200	...	50
Sclerotium bataticola	[c]	[c]	...	[c]

[a] Carried out at the Lilly Research Laboratories by F. Komann.
[b] The agar dilution technique was used, the bacteria being observed for forty-eight hours, and the bacterial and fungal plant pathogens for seventy-two hours.
[c] Test organism did not grow sufficiently well to establish an end point. Where no inhibitory concentration is reported, the organism was not affected by 200 μg./ml.

vious investigation (6), namely 1,2,5,6-di-O-isopropylidene - 3 - (2 - benzyloxy - 4 - nitrobenzoyl)-D-glucofuranose, monoacetone glucose, and diacetone glucose, were found to be devoid of activity in the antimicrobial screening test employed.

It may be concluded from these comparisons that the introduction of a glucose residue into a nitrobenzene derivative generally lowers the acute toxicity to a considerable extent. The relative position of the nitro group in a mononitro compound appears to have little effect on the toxicity. The antimicrobial activity of nitrobenzene derivatives was found to undergo considerable change with the introduction of a detoxifying group, and this activity is apparently a structurally specific effect and not a general one for nitro aromatics. The combination of nitro and halogen groups in the same molecule gave a superior antibiotic spectrum to that of molecules containing either nitro or halogen groups alone.

TABLE IV.—ANTIBIOTIC SPECTRA OF NITROPHENYL GLYCINES[a]

Test Organism	Inhibitory Concentration, μg./ml.[b] Compd. No. 9	11	12	13	14
Mycobacterium phlei	...	...	200	...	...
Mycobacterium tuberculosis	...	...	200	200	...
Mycobacterium avium	...	...	200	200	...
Agrobacterium tumefaciens	...	...	200	...	...
Xanthomonas malvacearum	200	200	200	200	...
Xanthomonas phaseoli	...	...	...	200	...
Corynebacterium insidiosum	...	200	...	...	200
Corynebacterium sepodonicum	200	200	200	200	200
Aspergillus niger	...	...	200	...	...
Ustilago avenae	200	200	200	200	200
Alternaria oleracea	200	200	200	200	200

[a] Carried out at the Lilly Research Laboratories by Sue Burden.
[b] The same footnote applies here as for Table III.

METHODS OF PREPARATION

The D-glucamine derivatives were obtained from the reaction of D-glucamine with nitroaryl halides, such as 2,4-dinitrochlorobenzene and 1,2-dichloro-4,5-dinitrobenzene. This reaction has been described previously (8), and was found to give primary aromatic amines more often than D-glucamine derivatives, but it was possible to obtain the N-4-nitrophenyl, N-2,4-dinitrophenyl, and N-2-nitro-4,5-dichlorophenyl D-glucamines by this method using either pyridine or ethanol-sodium acetate as solvent.

Preparation of the nitrophenylglycines was first attempted with the reaction of the respective nitrophenyl chlorides with glycine in the presence of pyridine. The reaction mixture, after refluxing, showed considerable decomposition, and pure products were not isolated.

Reaction of nitroanilines with chloroacetic acid in aqueous sodium acetate solution, however, gave low yields of products which were increased by varying reaction conditions. Isolation and purification of the crude products was achieved by conversion to the water-soluble sodium salts and reconversion to the insoluble acids, followed by recrystallization. The nitrophenylglycines were easily converted to ethyl esters.

EXPERIMENTAL

The melting points were taken on a Fisher-Johns block which gave correct values for a set of U. S. P. melting point standards. Analyses were carried out at the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

The preparation of D-glucamine, N-(4-nitrophenyl)-D-glucamine, and N-(2,4-dinitrophenyl)-D-glucamine have already been reported (8). The preparation of N-(2-nitro-4,5-dichlorophenyl)-D-glucamine was carried out according to the method of Holly, *et al.* (9), and a 52% yield of product melting at 188–191° was obtained (Holly reported a m. p. of 193–195°).

p-Bromobenzamide.—*p*-Bromobenzoic acid was prepared in 82% yield using the method of Chiang and Tseng (10); m. p. 246–250° (lit. (10) m. p. 251–253°). *p*-Bromobenzamide was prepared by conversion of *p*-bromobenzoic acid to the acyl chloride using a large excess of thionyl chloride according to the procedure of Meyer (11). This was then converted to the amide using concentrated ammonium hydroxide according to the method of Schotten (12). After recrystallization from aqueous ethanol, the overall yield from the acid was 47%; m. p. 186–188° (lit. (12) m. p. 189°).

N-(4-Bromobenzoyl)-D-glucamine.—D-Glucamine (10.0 Gm., 0.055 mole) and *p*-bromobenzoic acid (5.0 Gm., 0.025 mole) were placed in a flask with 15 ml. of anhydrous pyridine. The mixture was refluxed for eighteen hours, and the resulting solution was diluted with 15 ml. of water and steam distilled until 250 ml. of distillate was collected. The remaining solution was concentrated to about 20 ml. and chilled. A small quantity of precipitate was recrystallized from methanol to give 0.2 Gm. of white powder, m. p. 207–208°. This material gave a positive test for halogen and was not soluble in sodium hydroxide solution. Repeated recrystallization from methanol failed to raise the m.p. appreciably.

Anal.—Calcd. for $C_{13}H_{18}NO_6Br$: C, 42.87; H, 4.98. Found: C, 42.85; H, 5.02.

N-(o-Nitrophenyl)-glycine.—A mixture of *o*-nitroaniline (16.0 Gm., 0.116 mole), sodium acetate trihydrate (50.0 Gm., 0.368 mole), and water (200 ml.) was heated to boiling, and a solution of 32 Gm. (0.339 mole) of chloroacetic acid in 60 ml. of water was introduced, dropwise, over a period of two hours. Heating and stirring were continued for another hour, and the solution was adjusted to a pH of 9 using ammonium hydroxide solution. After cooling overnight, the reaction mixture was filtered, the filtrate acidified, and the precipitate collected. The residue first collected was extracted with 4% ammonia, and the extracts were acidified. The resulting precipitate was combined with the previous product from acidification, and the combined product was dissolved in aqueous ammonia, filtered, and reprecipitated with acid. The product was washed repeatedly with water, and further purified by reprecipitation and recrystallization from hot water to give 1.65 Gm. (7%) of deep orange platelets, m. p. 196–199° (lit. (13) m.p. 192–193°).

Ethyl N-(o-Nitrophenyl)-glycinate.—A mixture of 1.8 Gm. (0.009 mole) of N-(*o*-nitrophenyl)-glycine, 15 ml. of absolute ethanol, and 1 ml. of concentrated sulfuric acid was refluxed for four hours. A portion of the alcohol was distilled, an excess of aqueous ammonia was added to the cooled residue, and the resulting solid was filtered and washed with dilute ammonia and water. Recrystallization from absolute ethanol gave 2.0 Gm. (97%) of fine yellow needles, m. p. 80–81°.

Anal.—Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40. Found: C, 53.65; H, 5.65.

N-(m-Nitrophenyl)-glycine.—This compound was prepared by the same procedure as used for the *ortho* isomer. A 47% yield of yellow crystalline powder was obtained, m. p. 155–159° (lit. (14) m. p. 158–159°).

Ethyl N-(m-Nitrophenyl)-glycinate.—This ester was prepared by the same procedure as used for the *ortho* isomer. A 72% yield of golden platelets was obtained, m. p. 86–88° (lit. (15) m. p. 84°).

N-(p-Nitrophenyl)-glycine.—The same general procedure was used as for the *ortho* isomer. A 32% yield of yellow, crystalline powder was obtained, m. p. 230–232° (lit. (14) m.p. 225°).

Ethyl N-(p-Nitrophenyl)-glycinate.—The same procedure was used as for the *ortho* isomer. A 96% yield of light yellow needles was obtained, m. p. 138.5–140°.

Anal.—Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40. Found: C, 53.33; H, 5.42.

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A Note on the Preliminary Phytochemical and Biological Study of *Lomatium Suksdorfii**

By FRANK A. PETTINATO†, LOUIS FISCHER, and NATHAN A. HALL

Lomatium Suksdorfii (Watson) Coult. and Rose, *Umbelliferae*, grows along the Columbia River in the state of Washington and is one of about 80 species of *Lomatium*.[1] The roots of several species were used medicinally by the Gosiute Indians who called the plant "pia-a-na-tsu" or "great medicine" (2). The roots of two species have been reported to yield oils and extractives which were active antibacterials *in vitro* against numerous microorganisms (3–5). In the fuits of *L. Suksdorfii*, Call (6) has found an oil and a crystalline substance which was an active spasmolytic.

The work reported here was undertaken in preparation for a more exhaustive study of the components of the fruits and roots of the plant. The plant material was collected in July 1956 near Klickitat, Washington, dried in air, and ground with a Wiley mill (1-mm. screen) immediately before use. The ground fruit and root were submitted to proximate analysis (see Table I). Both plant parts, when subjected to a glycoside extraction technique (7) produced a small amount of crystalline residue which was not further characterized. All tests for alkaloids were negative.

TABLE I.—PROXIMATE ANALYSIS OF *L. Suksdorfii*

	Root, %[a]	Fruit, %[a]
Moisture	7.8	7.0
Total ash	9.7	7.7
Acid insoluble ash	2.3	0.30
Reducing sugars (as invert sugar)	1.5	Trace
Sucrose	3.6	2.8
Starch	11.6	11.0
Nitrogen (Kjeldahl)	0.83	0.31
Protein (N × 6.25)	5.2	2.5

[a] Based on air-dried weight.

The ground root on steam distillation yielded 0.39% volatile oil and the ground fruit yielded 3.7% volatile oil. Determination of the physical constants and chemical analysis of the fruit oil gave the results shown in Table II.

TABLE II.—PHYSICAL AND CHEMICAL PROPERTIES OF *L. Suksdorfii* OIL

Specific gravity, 25°/25°	0.8435
Refractive index, 23°	1.4808
Optical rotation, 25°	47.50
Acid number	0.42
Ester number	3.5
Ester number (after acetylation)	13.4
Esters (as bornyl acetate)	1.2%
Alcohols (as borneol)	3.7%
Aldehydes and ketones (by neutral sulfite method)	4.5%
Aldehydes and ketones (by hydroxylamine method, assuming average M. W. of 150)	2.2%

The fruit oil was tested for antibacterial activity against growing cultures of *Micrococcus aureus* var. *pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus pyogenes*, and *Proteus vulgaris* by the filter paper disk method of Vincent and Vincent (8). Simultaneous comparisons were made with cinnamon, eucalyptus, and rectified turpentine oils. *L. Suksdorfii* oil exhibited no significant activity against any of the organisms except *B. subtilis* and *S. pyogenes*, and in these cases the activity was no greater than the oils used for comparison.

The volatile oil from the fruit was also screened *in vitro* for anthelmintic activity against *Ascaris lumbricoides* of swine by the Lamson and Brown procedure (9). When it was emulsified with 0.2% acacia, *L. Suksdorfii* oil was ascaricidal in 4% concentration after a two-hour exposure period. Much greater activity was shown by other anthelmintics used for comparison; hexylresorcinol (0.1%) was ascaricidal after a two-minute exposure and chenopodium oil was ascaricidal after a ten-minute exposure.

Detailed investigation of the oil from the fruit will be reported in a subsequent paper.

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[1] Considerable confusion regarding the genus *Lomatium* exists in the literature. Although the term Lomatium is currently accepted, the genus has been variously designated as *Peucedanum*, *Cogswellia*, and *Leptotaenia* (1). Specimens were identified by Dr. A. E. Kruckeberg, University of Washington and Dr. Lincoln Constance, University of California, whose assistance is gratefully acknowledged.

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A Note on the Use of Oxygen and Carbon Dioxide in Perfusion Fluids*

By G. H. BRYAN and G. R. SHUCK

REPORTS of the perfusion of isolated mammalian hearts with artificial fluids is complicated by the use of various gases, as either oxygen alone or in combination with 1 or 5 per cent CO_2 (1). The two factors, the final oxygen concentration and pH, to which the tissue is exposed may lead to discrepancies in duplication of experimental results.

In an extensive theoretical and applied treatment, Ling and Smith (2) have described the problem of pH control by gases in various perfusing fluids and have suggested a method of achieving a specific pH.

The oxygen content has proved important in this laboratory and consequently it was of interest to find a procedure which would permit oxygen supersaturation and a final pH of 7.4.

METHODS

Locke-Ringer's U.S. P. XV (3) solution was used. Ten liters were freshly prepared each time the solution was needed. To facilitate electrical stirring, pH measurement, and sample withdrawal, a 15-L. battery jar was used as the container. Oxygen and 5% carbon dioxide in oxygen were delivered through gas dispersion tubes (coarse) submerged to 20 cm. and mounted on the side of the jar. Gas flowed through a 5-mm. rubber tube under 67 mm. of mercury gauge pressure. This corresponds roughly to a vigorous but not turbulent flow. Oxygen determinations were made by the Rideal Stewart modification of the Winkler method (4). So that monitoring of pH could be continual, four-foot extensions were spliced into the electrode systems of a Beckman pH meter, Model H2.

RESULTS

Twelve one-hour series of oxygen determinations at ten-minute intervals from the onset of oxygen gassing revealed that a near-maximum saturation was reached in twenty minutes. This was found with Locke-Ringer's solution at 37, 26, and 10°. After the gas flow and stirring was stopped, equilibration with the air did not affect the oxygen content of Locke-Ringer's solution gassed at 26 and 10° for approximately two hours. Therefore, with this fixed oxygen flow, a lesser time of gassing does not allow saturation to be reached and a greater time wastes oxygen.

If a physiological pH is considered to be 7.4, then it was of interest to monitor the pH resulting from oxygen gassing. From a starting value of 7.9 the pH was 8.6 at the end of twenty minutes. Therefore, the condition of pH 7.4 cannot be met with oxygen gassing of Locke-Ringer's solution. This is in general agreement with Ling and Smith (2) for other fluids and for the sodium bicarbonate content. Gassing in the same manner with 5% carbon dioxide in oxygen resulted in a pH shift from a starting value of 7.8 to 6.7 after twenty minutes of gassing. Therefore, the condition of pH 7.4 cannot be met with 5% carbon dioxide in oxygen gassing.

As a result of these findings with Locke-Ringer's solution it was necessary to gas with oxygen for twenty minutes to obtain saturation, then shift to gassing with 5% carbon dioxide in oxygen until a pH of 7.4 had been obtained. Over and undershooting could be reversed with the appropriate gas.

Variations exist in the ultimate delivery of perfusion fluids. Tissue may be placed in the container in which the fluid was prepared or the fluid may be moved to the tissue through a series of glass or rubber tubes. There also exists the possibility of a temperature gradient from a stock container to the tissue. In this instance, it was desired that the fluid be pumped from a stock container to a reservoir 80 cm. above the table top, allowed to flow through a constant temperature bath (1,000 watt heater) at 37°, and then be delivered to an isolated heart suspended 125 cm. below the reservoir. Oxygen values found at the start and finish of this route and at three different starting temperatures are shown in Table I. From Table I, it may be seen that there is a gain in oxygen at the delivery end of the system if the initial gassing is conducted at temperatures lower than 37°. Whether the gain at lower temperature is important or not will depend upon tissue requirement.

As a practical matter, storage of gassed solutions for twelve hours in filled, rubber-stoppered containers at ice box temperatures has been satisfactory.

SUMMARY

It is recommended that perfusion fluids of the Locke-Ringer type be gassed with oxygen at temperatures lower than 37° for twenty minutes and the pH adjusted by gassing with 5% carbon dioxide in oxygen. The solution may then be stored or used within a two-hour period.

TABLE I.—SOLUBILITY OF OXYGEN[a] IN LOCKE-RINGER'S SOLUTION AT VARIOUS TEMPERATURES UNDER 673 MM. OF HG PRESSURE

	Dissolved oxygen in parts per million by weight		
Temp., °C.	A	B	C
37	22.2	21.0	21.0
26	31.0	29.0	25.0
10	42.4	38.0	28.0

A—O_2 Content after twenty minutes of gassing.
B—O_2 Content after additional gassing with 5% CO_2 in O_2.
C—O_2 Content after pumping solution from stock container and through a perfusion system.
[a] The above values are approximately four times the equilibrium value listed by Griffin (4) due to supersaturation.

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* Received April 18, 1957, from the School of Pharmacy, Montana State University, Missoula.

A Note on the Stability of Vitamin B_{12} in the Presence of Thiochrome*

By LOUIS J. RAVIN and ROBERT F. DOERGE

In a recent review article Scheindlin (1) discusses the chemical interactions of the water-soluble vitamins. He points out that while several studies have been published (2–6) concerning the stability of vitamin B_{12} in the presence of thiamine and nicotinamide, much remains to be learned about the reaction between thiamine and nicotinamide, as well as the reaction between thiamine and vitamin B_{12}. In addition to the references cited by Scheindlin (1), additional information on this problem has been reported by Gambier and Rahn (7, 8). Several of these studies have implicated the thiazole moiety of thiamine in the breakdown of vitamin B_{12}, however, no mention has been made concerning the possible role of thiochrome. This note presents some preliminary data regarding the possible interaction of thiochrome, an oxidation product of thiamine, with vitamin B_{12} in aqueous buffered solution.

EXPERIMENTAL

Thiochrome obtained from Bios Laboratories Inc. and cyanocobalamin U. S. P. (crystalline vitamin B_{12}, Merck and Co.) were used in these studies.

Solutions containing 25 μg. of crystalline vitamin B_{12} and 8.5 mg. of thiochrome[1] in each 5 ml. were prepared and adjusted to pH 4.0. A control solution containing 25 μg. of crystalline vitamin B_{12} was also prepared. The finished solutions were filtered through a Selas[2] unglazed porcelain candle, 02 porosity, and packaged into 1-oz. amber bottles. Samples were set down at RT, 45, 60, and 85° and assayed periodically for vitamin B_{12} by the U. S. P. microbiological method (9).

DISCUSSION

The assay data obtained in this study are summarized in Table I.

Table I.—Stability of Crystalline Vitamin B_{12} in Aqueous Buffered Solution Containing Thiochrome*

Storage Condition	Storage Time	Solution Containing Thiochrome, μg./5 ml.	Control, μg./5 ml.
Original	.....	28	28
85°C.	8 hours	28	28
	16 hours	26	27
	24 hours	26	26
RT	3 weeks	29	29
45°C.	3 weeks	27	30
60°C.	3 weeks	29	29
RT	7 weeks	29	26
45°C.	7 weeks	27	24
60°C.	7 weeks	25	24
RT	3 months	27	28
45°C.	3 months	27	26
60°C.	3 months	23	22

* 8.5 mg./5 ml., equivalent to 11 mg./5 ml. thiamine hydrochloride.

The above data clearly indicate that thiochrome is not responsible for accelerating the decomposition of vitamin B_{12} in an aqueous buffered solution under these experimental conditions. Additional studies concerning the interaction between thiamine and vitamin B_{12} which also include the possible reaction with thiamine decomposition products are in progress. A complete report on the results of these studies will be forthcoming.

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* Received September 20, 1958, from the Research and Development Division of Smith Kline and French Laboratories, Philadelphia 1, Pa.
[1] Equivalent to 11 mg./5 ml. thiamine hydrochloride.
[2] Selas Corporation of America, Philadelphia, Pa.

Book Notices

Handbook of Toxicology. Vol. IV. Tranquilizers. Edited by Rudolph M. Grebe. W. B. Saunders Co., West Washington Square, Philadelphia, Pa., 1959. viii + 120 pp. Paperbound. Price $4.

This handbook is the eighth of a continuing series of publications, each containing information, chiefly tabular, in one or more of the biological sciences. All of the volumes in the series have been prepared under the general direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, National Academy of Sciences National Research Council. This latest volume furnishes, in outline form, pertinent data on 26 tranquilizers which have been used as psychopharmacological agents. In general, the information about each of the drugs in this class includes the nonproprietary name, the trademark, the name of the manufacturer, the molecular formula and weight, the structure, a brief description of the physical and chemical properties, the pharmacology, certain clinical data, the toxicity, and the mode and site of action. The book is documented with the more important references to the original literature. The book is well designed to furnish information in summary form on the principal tranquilizers now being used.

Übersicht der gebräuchlichen und neueren Arzneimittel. By E. BERNOULLI and H. LEHMANN. Benno Schwabe & Co., Basel, Switzerland, 1959. U. S. Representative, Intercontinental Medical Book Corp., New York. xlviii + 555 pp. 12 x 18 cm. Price $5.75.

This relatively small book contains enormous amounts of information in outline form, concerning newer medicinal chemicals as well as many of the older, but still widely used drugs. The information presented is logically classified according to the therapeutic categories of the various drugs discussed. The book is particularly helpful in locating information on the nature of German and certain other European trademark names and nonproprietary names of drugs. Chemical and physical properties of the drugs covered are usually presented, and a list of available dosage forms are often given. The book is provided with an unusually complete index which facilitates locating needed information easily and promptly.

Hagers Handbuch der Pharmazeutischen Praxis. In 2 vols. Edited by WALTER KERN. Springer-Verlag, Berlin, 1958. xvi + 2,544 pp. 16.5 x 24 cm. Price DM 224.

A complete revision of this well-known reference book on the practice of pharmacy is now available in two volumes, the first covering the alphabet A–H and the second I–Z. The two volumes are paged consecutively, and the second volume contains the index for both. The new edition continues to be a comprehensive and thoroughly reliable reference for many pharmaceutical fields of endeavor. A more complete review of the new edition of Hager appears in THIS JOURNAL, *Pract. Pharm. Ed.*, **20,** 342(1959).

The Profession of Pharmacy. By RICHARD A. DENO, THOMAS D. ROWE, and DONALD C. BRODIE. J. B. Lippincott Co., East Washington Square, Philadelphia, Pa., 1959. xii + 256 pp. 18 x 25.5 cm. Price $6.50.

This introductory textbook is unique in character and different from any other textbook that has ever been written. It provides the beginning student in pharmacy with a broad coverage of the profession including its historical development, ethical standards, organizations, literature, and current problems. Its organization covers the major fields of pharmaceutical practice including pharmaceutical education, retail pharmacy, pharmaceutical research, manufacturing pharmacy, promotion and distribution of drugs, hospital pharmacy, development of standards for drugs, and activities involving pharmaceutical legislation. Each chapter is followed by provocative study questions and a list of selected references for additional reading. The scope of the book can best be visualized from the titles of the fifteen chapters which are: Pharmacy and other health professions; Birth of a drug; Pharmaceutical education; Roots of retail pharmacy; Modern retail practice; Organizations and periodicals of retail pharmacy; Nature and climate of science; Pharmaceutical research; The pharmaceutical industry; Production and quality control; Promotion and distribution of drugs; Hospital pharmacy; Pharmacopeias and other standards; Legal regulation; Pharmacy—past and future. Interest in the text is enhanced by the inclusion of 85 well-chosen illustrations of the subject matter discussed. The design of the book and the presentation of the subject matter are excellent throughout, and reflect the thought and care of the authors that must have gone into its preparation. It should be well received, and probably will be adopted as a standard textbook in many colleges of pharmacy.

Précis de Chimie Générale et de Chimie Minérale Book I. By L. DOMANGE. Masson et Cie., boulevard Saint-Germain, Paris 6e, France, 1959. 305 pp. 16 x 21.5 cm. Price 2,200 fr.

This book (in French) is a concise review of general and inorganic chemistry intended for use as a textbook for one semester in a course for beginners. It is designed as part of a series of textbooks for pharmacy students.

Principles of Microbiology. 2nd ed. By WALTER H. KRUEGER and KARL R. JOHANSSON. W. B. Saunders Co., West Washington Square, Philadelphia 5, Pa. 1959. xxiv + 563 pp. 15.5 x 23.5 cm. Price $6.75.

The second edition of this textbook has been completely revised by the inclusion of new developments and points of view in the science of microbiology. The general organization of the previous edition is a retained, but a substantial amount of new material and several new illustrations have been introduced. The book is intended primarily as a textbook for an introductory survey course in general microbiology for college students.

Index of Medicines Produced in Israel. By J. ALADJEMOFF. F. Pinczower, Tel-Aviv, Israel, 1953. Available in the U. S. from J. Pincover, 602 West 157th St., New York 32, N. Y., 492 pp. 12 x 16.5 cm.

This book lists alphabetically drugs produced in Israel, including the dosage forms, indications for use, dosage, and other pertinent information. The list indicates that the pharmaceutical and chemical industry in Israel is growing and developing rapidly. This may be due in part to restrictions on imports of drugs produced in other countries. The book is designed to assist physicians in choosing the proper preparation, ascertaining its dose, and the directions for its administration.

Selected Papers from the Institute of Cancer Research: Royal Cancer Hospital and from the Royal Marsden Hospital. Vol. II. Lund Humphries, London, 1958. xix + 928 pp. 19.5 x 26.5 cm.

A collection of reprints of ninety-eight papers relating to chemical, pharmacological, and clinical aspects of cancer published in scientific journals during 1956. This compilation should serve as a very great convenience to any worker in cancer research by having available in one volume such a large number of significant published reports.

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